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ANGIORETICULOENDOTHELIOMA (KAPOSI'S DISEASE) OF THE HEART *

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"There develop on the skin, without known cause either general or local, brown-red to blue-red nodules of the size of a grain of wheat, a pea or a hazel nut. Their surface is smooth, their consistence densely elastic. Often they are swollen like a sponge filled with blood." So in 1872 wrote Kaposi [31] describing the lesions seen in a disease which has since borne his name. Subsequent study of some 25 cases of the disease enabled him to deduce certain generalizations regarding the entity. The lesions are first observed on the extremities, subsequently they appear on the face and trunk. Spontaneous regression of some of the nodules is a characteristic feature, but no type of treatment ever affords more than temporary relief. Two to 5 years after onset visceral metastases begin and the disease terminates fatally in 3 to 8 years.

Following Kaposi's observations dermatologists throughout the world have studied and recorded cases in which a great variety of manifestations have been observed. On the basis of experience the disease is no longer regarded as invariably fatal and visceral metastases are anticipated in only about 10 per cent of cases. Microscopic examination of the lesions has led to fairly general agreement as to the histological features necessary for establishment of the diagnosis. Nevertheless, there is still but little agreement regarding the etiology and pathogenesis of the condition.

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TABLE I

Digest of Cases Reported in Literature Since 1932

Author	Number of cases	Sex	Age	Race or nationality	Occupation	Duration of lesion	Location of lesion	Microscopic diagnosis	Special features	Animal inoculation	Group	Theory of nature of disease
			yrs.									
Andrews [1]	1	M	19	Mulatto	Truck driver	10 wks.	Skin	(1) Fibrosarcoma (2) Kaposi				Not stated
Arias [2]	1	M	49	Spanish		6 mos.	Skin	Kaposi				Not stated
Barringer and Dean [4]	2	M	45			— mos.	Skin and penis	Kaposi			1	"This case supports neuro-origin"
		M	56	Italian		2 mos.	Skin and penis	Kaposi				
Bilancioni [6]	1	M	62	Italian	Merchant	12 yrs.	Skin and larynx	Kaposi			1	Systemic vascular disease with neoformation of caps.
Carpino and Secchi [7]	1	M	58	Italian	Sponge fisher	8 mos.	Skin	Kaposi	Antecedent fibrosarcoma, died		1	"More neoplastic than a granuloma"
Casabianca *et al.*[8]	1	M	52	Corsican	Sailor	6 mos.	Skin	Kaposi			4	Reticuloendothelial hyperplasia
Van Cleve and Hellwig [10]	1	M	58	American	Painter	2 yrs.	Skin, liver, kidneys, stomach, intestine, pericardium	Kaposi	1st lesion nodule in groin, died		3	Granuloma becomes neoplastic
Denzer and Leopold [13]	1	M	4	Italian		1 yr.	Skin, widespread, internal	Kaposi	Died			Not stated
Dupont [14]	12		Case histories not given					Kaposi	20 biopsies		4	Angioreticulosis *cf.* von Recklinghausen's neurofibromatosis
Eljasz [15]	1	M	76	Polish Jew		17 yrs.	Skin and bladder	Kaposi	Cornu cutaneum superimposed			Not stated
Ellis [16]	1	M	40	American negro		2 yrs.	Skin	Kaposi	In blood smear 46% lymphocytes			Not stated
Frölich [18]	1	M	44	Polish Jew	Ditch digger	1½ yrs.	Skin	Kaposi			4	Reticuloendothelial disease

Goldschlag [19]	1	M	65	Russian Jew	Merchant	3½ yrs.	Skin and viscera (?)	Kaposi	Antecedent lymphogranuloma		4	Reticuloendothelial disease
Gonzales and Vidaurreta [20]	1	M	55	Italian		1½ yrs.	Skin	Kaposi	Died		1	Angiosarcoma
Gougerot *et al.*[21]	1	F	57	Armenian		9 yrs.	Skin	Kaposi				Not stated
Gougerot *et al.*[22]	1	M	39		Wine merchant	2 mos.	Skin and mouth	Kaposi				Not stated
Greppi and Bettoni [23]	1	M	40			1 yr.	Penis, lymph node, psoas, lung	Kaposi	Hemolytic splenomegaly, died		4	Chronic reticuloendotheliosis
Grzybowski [25]	3	M	54	Jew		12 yrs.	Skin		Died		1	Neoformation of blood vessels with endothelial hyperplasia
		F	46	Jew			Skin					
		M	63	Jew		16–20 yrs.	Skin and tongue	Kaposi				
Guccione [26]	2	M	56			6 mos.	Skin	Kaposi	Died		4	Angioreticulosis with possible endocrine basis
		M	63			1 yr.	Skin	Kaposi				
Homma [28]	1	M	70		Laborer	10 yrs.	Skin	Kaposi			2	Specific infection
Jessup [29]	1	M	65	Italian		11 mos.	Skin	Kaposi				Not stated
Kusnezow [24]	1	M	32	Caucasian		2 yrs.	Skin, tongue, trachea, intestine	Kaposi	In blood smear 55% lymphocytes, died			Visceral lesions not metastases
Lane and Greenwood [35]	1	M	42	Italian	Laborer	3 yrs.	Skin	Kaposi	Also lymphatic leukemia and mycosis fungoides, died			Possible common etiological factor for all

TABLE I (*Continued*)

Author	Number of cases	Sex	Age	Race or nationality	Occupation	Duration of lesion	Location of lesion	Microscopic diagnosis	Special features	Animal inoculation	Group	Theory of nature of disease
			yrs.									
Lang and Haslhofer [36]	2	F	58		No further data			Kaposi			1	Generalized hemangiomatosis with anomaly in 1st anlage of vascular system
		M	51		No further data			Kaposi				
Leigheb [37]	1	M	52	Italian		1 mo.	Skin	Kaposi	Cardiovascular renal disease		Miscellaneous	"Affection of vascular system"
Mierzecki [40]	7	M	26	Jew		2 yrs.	Skin, lymph node, lungs, kidney					
		M	62	Jew		Many yrs.	Skin				1	Starts as neoplasm, ends as simple granulation tissue
		M	29	Jew		6 mos.	Skin					
		M	65	Greek catholic	Carpenter	2 yrs.	Skin	Kaposi				
		M	74	Greek catholic	Watchman	3 yrs.	Skin					
		M	62	Jew	Woodman	2 yrs.	Skin					
		M	46	Roman catholic	Bricklayer	24 yrs.	Skin and bones	Kaposi				
Pack [42]	1	M	72	Russian Jew		3 yrs.	Skin	Kaposi		Reinoculation negative	1	Glomus type of tumor
Pardo Castello [44]	1	M		Negro	(Quoted by Ellis)					No further data		Not quoted
Pearce and Valker [46]	1	M	50			6 mos.	Skin, gum, genitalia	Kaposi	Died		1	Sarcoma
Pitotti [47]	1	M	17	Italian		2 yrs.	Skin	Kaposi				Not stated

Roger and Vigne [48]	1	M	50	Corsican	Navigator	9 yrs.	Skin, lips, genitalia	Kaposi	Von Recklinghausen's disease in bones, died, no autopsy			Not stated
Cantori [49]	4	M	50	Italian		1 yr.	Skin	Kaposi				
		M	59	Italian		3 yrs.	Skin	Kaposi		Anterior chamber eye of guinea pig negative	Miscellaneous	"Systemic vascular disease"
				(Same case as Bilancioni's)					Died, no autopsy			
		M	70	Italian		25 yrs.	Skin	Kaposi				
Schirmunskaja and Tschotschia [50]	1	M	22	Russian	Clerk	5 yrs.	Skin, pharynx	Kaposi			1	Benign tumor
Sézary and Bardin [51]	1	M	80			4 yrs.	Skin	Kaposi	Original diagnosis von Recklinghausen's disease			Not stated
Vigne and Pédat [54]	1	M	63	Corsican	Docker	2½ yrs.	Skin	Kaposi				Not stated
Webster [56]	1	M	77	Polish Jew		6 yrs.	Skin	Kaposi	Died of pneumonia, no autopsy			Not stated
Thoisser and Ramsey	2	M	26	American	Clerk	2 mos.	Heart, lungs, liver, pericardium	Kaposi	Died	Negative	4	Angioreticuloendothelioma
		M	30	American	Policeman	1 mo.	Heart	Kaposi	Died	Negative	4	Angioreticuloendothelioma

For this reason it is important that each new case encountered be regarded not only on its merits but also in the light of other cases reported in the literature. Two cases which we have recently been privileged to study clinically and at postmortem present certain features not previously described and stress the fact that Kaposi's disease is one of general medical importance as well as of dermatological significance.

Review of the Literature

Two recent monographs have dealt exhaustively with the older literature on the subject and for our present purposes it is sufficient to adopt the statistics compiled by their authors rather than to refer directly to original reports, each dealing with but a few isolated cases. Dörffel [13] examined the literature up to 1932 in connection with a report of 16 cases which had come under his own observation. Kren [33] wrote the chapter on Kaposi's disease in Jadassohn's Handbuch der Haut- und Geschlechtskrankheiten in the following year. This very detailed treatise provides a complete bibliography and thorough analysis of all cases reported and all theories pertaining to etiology, as well as discussions of the pertinent clinical and therapeutic problems.

The literature subsequent to 1932 has been reviewed by the present writers and the figures gleaned from it are added to those of Dörffel and Kren to form the basis of Tables I, II and III, and Chart 1, which carry the statistics down to the end of the year 1937. Data from the 2 cases herewith reported are also included in the statistical summaries.

Age Incidence: Dörffel gathered 356 cases from the literature and added 16 of his own. Of this total of 372 cases the age was stated in 234. Our review of subsequently published cases has added 60 to the total, in 47 of which the age is known. The age in the 2 cases herewith reported is also known. Chart 1 shows the age distribution, by decades, of the total 283 cases. The peak of incidence in the 5th, 6th and 7th decades has been recognized as a characteristic finding throughout the history of the disease.

Sex: Dörffel counted 21 females among the 356 cases in the literature, and 2 of his own cases occurred in women. Kren considers this figure too low as his count of "nearly 500 cases" included "about 40 females." Among the cases recorded since 1932

we found 3 women. Those herewith reported occurred in men. In our series from the literature, as in Dörffel's, the sex of the patients is not always ascertainable. Combining the known cases from both series, the ratio of male to female is found to be 385:26.

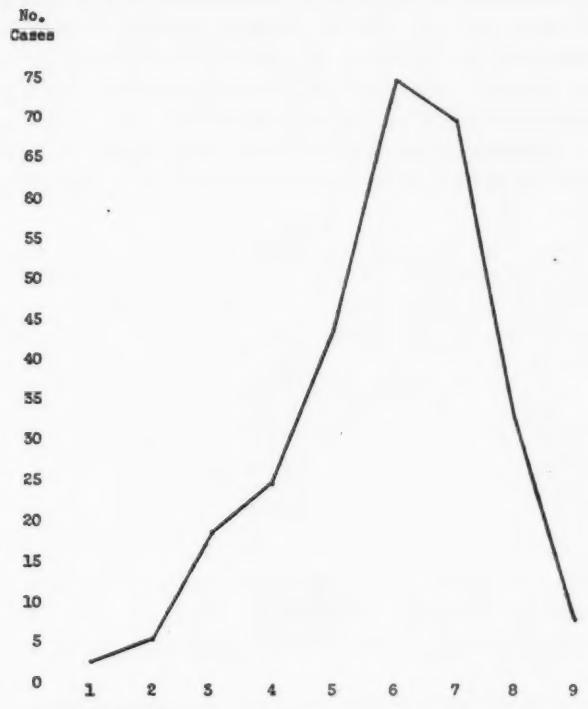

Chart I

Age Incidence of Kaposi's Disease
Total 434 Cases

Thus, women represent 5.99 per cent of the (uncorrected) total of 434.

Race: The racial stock or nationality of the patient is given in 40 of the 62 recent cases (including our own 2). The distribution is as follows: 12 Italians, 12 Jews (2 Russian, 3 Polish, 7 unspecified), 3 Corsicans, 3 Americans, 2 negroes (1 Cuban, 1 American), 1 "North Caucasian," 1 mulatto, 1 Spanish, 1 Armenian,

1 Russian, 2 "Greek catholics," 1 "Roman catholic." This is in line with Dörffel's opinion that the disease is more closely linked with geographic than with racial origin, Eastern Europe and Northern Italy being the areas where most of the cases originate, though no part of the world lacks representation, with the possible exception of China and Japan (Mainta, quoted by Kren).

Occupation: The patient's occupation is recorded only 17 times in recent reports. Outdoor labor is noted 13 times (truck driver, painter, laborer, sailor, fisherman, carpenter), indoor work 4 times (clerk, merchant, wine merchant). In addition, of our 2 patients 1 was a policeman and the other a mailing clerk. This agrees with

TABLE II

Location of Visceral Lesions as Recorded in Literature Prior to 1932

Most common (in order)	Frequent	Uncommon	Rare	Never recorded
Gastro-intestinal tract, all portions	Faucial tonsil	Spleen	Heart	Brain
Liver	Bones	Pancreas	Pericardium	Thyroid
Lungs	Larynx	Kidneys	Central nervous system	Ovary
Lymph nodes retroperitoneal mesenteric	Conjunctiva	Adrenals	Peripheral nerves	Uterus
		Peritoneum	Tongue	
		Testes	Bladder	
		Epididymis	Muscles	
		Trachea	Pituitary	
		Bronchi		
		Pleura		

Dörffel's and Kren's finding that office workers and professional men are less frequently affected than laborers and out-of-door workers. The discrepancy is more pronounced in their larger series.

Duration of Disease: The cases in the recent literature all fall within the general limits noted by Dörffel, namely, 8 months to 25 years where the disease was fatal. Six months to 2 years is the spread in the 13 fatal cases recorded in the literature since 1932. Our own 2 patients form an exception. One was ill for only 1 month and the other for 2 months.

Distribution of Visceral Lesions: Most authors take particular care to avoid the use of the term "metastasis" in this connection because of its specific connotation. Since there is no general agreement that the disease is truly a malignant neoplasm, it would be undesirable to designate the visceral lesions as metastatic. The accompanying table (Table II), an adaptation of summaries pre-

sented by Dörffel and Kren, shows the relative frequency of involvement of various viscera as reported in the earlier literature. For the most part the statistics are based on positive autopsy findings, although hemorrhages from the orifices in patients with cutaneous lesions were sometimes accepted as proof of visceral involvement. Table III shows the distribution of visceral lesions reported since 1932, including the cases herewith described. In this series, autopsy or biopsy findings confirmed the clinical diagnosis in every case except the one of urinary bladder involvement in which the diagnosis rested on cystoscopic examination of a patient whose skin lesion was examined microscopically.

TABLE III

Location of Visceral Lesions as Recorded in Literature Since 1932 (Authors' own cases included)

Location	Number of times	Location	Number of times
Penis	3	"Widespread" (not specified)	2
Oral cavity	3	Heart	2
Upper respiratory tract	3	Tongue	2
Lymph nodes	3	Pericardium	2
Lung	3	Tonsil	1
Stomach	2	Bladder	1
Intestines	2	Diaphragm	1
External genitalia (male)	2	Psoas muscle	1
Liver	2	Bones	1
Kidney	2		

Up to the time of Dörffel's and Kren's articles no case had been reported in which skin lesions were lacking, and only in Paolini's case [43] did a visceral lesion antedate the cutaneous manifestations. The literature since 1932 includes the report of 1 patient (that of Greppi and Bettoni [23]) in whom no skin lesion ever appeared during the year of illness prior to death. The primary lesion occurred upon the glans penis, the inguinal lymph nodes became involved, and at autopsy there was extension into the psoas muscle and nodules were found in the lung. Four reports in recent years have dealt with cases in which non-cutaneous manifestations preceded skin lesions. Pearce and Valker's [46] patient first noted a nodule on his gum several months before his skin became involved. Barringer and Dean [4] twice noted primary lesions on the glans penis. Van Cleve and Hellwig,[10] and Goldschlag [19] reported cases

in which the inguinal lymph nodes were the primary seat of the disease. In Goldschlag's case the original lymph node involvement was diagnosed lymphogranuloma inguinale, but he looked on this as an "ungewöhnlichem Vorstadium" of Kaposi's disease. The cases herewith reported lacked skin lesions and were primary in the right auricle of the heart.

Theories of Origin: There are nearly as many theories regarding the origin of this disease as there are investigators in the field. Briefly, the opinions may be classified in four major groups: (1) neoplasm; (2) infectious granuloma; (3) infectious granuloma with neoplastic potentialities; and (4) reticuloendothelial hyperplasia. For an exhaustive treatment of this aspect of the study of the disease the reader is referred to Kren's article. In the present connection it is desirable to mention only the most important subheadings in these four main groups to supply the necessary background for evaluation of recent opinions.

(1) Adherents of the theory of neoplasia are divided as to whether the tumor is benign or malignant and as to the origin and nature of the tumor cells. The nomenclature proposed indicates some of the opinions current on these points (Table IV).

(2) Proponents of the infectious granuloma theory have in their favor the natural history of the disease which so frequently includes spontaneous regressions which would be hard to reconcile with a malignant neoplasm. To their disadvantage stands the fact that no etiological agent has ever been demonstrated despite extensive bacteriological work, and that transfer of the disease to animals has never been definitely established, although attempts have been made using every common laboratory animal and bird. Reinoculation of patients with their own tumor tissue has also failed to give positive results (Pack [42]).

(3) The existence of certain "predisposing causes" and "exciting factors" is postulated by various authors to explain a malignant change in a previously benign growth, a neoplastic transformation on the part of an infectious granuloma or the development of reticuloendothelial hyperplasia. These include trauma, chilling or freezing of affected parts, alcoholism, drug sensitivity, erysipelas, cellulitis, arteriosclerosis, syphilis and acute systemic diseases, such as cholera, malaria, grippe and pneumonia.

(4) Those who consider the condition merely a reticuloendo-

thelial hyperplasia note not only the suggestive histological picture of the disease and the simultaneous occurrence of widely scattered lesions, but also the not uncommon occurrence of other stigmata of disease of the reticuloendothelial system in patients suffering from Kaposi's disease. Cases have been recorded in which the patient showed variations from mild lymphocytosis to

TABLE IV

Sarcoma Idiopathicum Multiplex Haemorrhagicum (Kaposi, 1872)

Synonyms

1. Angiosarcoma peritheliale fusocellulare
2. Angiosarcoma pigmentosum
3. Primitives haemorrhagisches acrosarcoid
4. Acrosarcoma multiplex cutaneum telangiectoides
5. Acroangioma hemorrhagicum
6. Sarcomatosis cutanea idiopathica
7. Sarcomatosis primitiva telangiectosica
8. Granuloma multiplex hemorrhagicum
9. Sarcoma cutaneum teleangiectaticum multiplex
10. Angioendothelioma cutaneum
11. Sarcoma haemangioendotheliale intravasculare
12. Haemangioendothelioma cutaneum
13. Sarcomatosis teleangiectaticum cutanea idiopatica generalisata
14. Perithelioma multiplex nodulosum cavernosum lymphangiectoides cutaneum
15. Acroperithelioma idiopathicum multiplex cavernosum lymphangiectoides cutaneum
16. Pseudosarcomatosis teleangiectaticum Kaposi
17. Sarcomatosis multiple hemorrhagica y pigmentaris tipo Kaposi
18. Sarcomatosi cutanea multipla emorragica di Kaposi
19. Multiple idiopathic hemorrhagic sarcoma of Kaposi
20. Angiosarcoma multiplex
21. Angiosarcoma endotheliale
22. Granuloma angiomatoides
23. Sarcoid Kaposi
24. Acrosarcoma Kaposi
25. Systematisierte angiomatosis
26. Kaposi's sarcoma
27. Kaposi's disease
28. Angioreticuloendothelioma

frank lymphatic leukemia, mycosis fungoides and lymphogranuloma inguinale.[11, 16, 19, 23, 34, 35, 55] The sites in which lesions of Kaposi's disease are found further support the hypothesis that it is of reticuloendothelial origin, since the regions most richly supplied with such tissue are most commonly affected.

The opinions of recent investigators on the disputed question of the origin and nature of Kaposi's disease are recorded in the last column of Table I. The numerical notations in the column headed

"Group" in that table refer to the four general groups noted above.

Case Histories

Case 1. A white male, 26 years of age, a mailing clerk, entered the hospital Nov. 8, 1937, complaining of generalized weakness, shortness of breath, and pain in the left arm. He had lived in the city of Washington, D. C. all his life. The family history and previous history were non-contributory. Two months before admission he had had a week's illness which was diagnosed acute influenza. Upon recovery he went back to work for 3 days but was forced to return to bed because of weakness and dyspnea. These symptoms became progressively more severe. Night sweats occurred and he developed a persistent pain in the left arm which radiated from the axilla to the finger tips.

Physical Examination: On admission the physical examination revealed a well developed man, acutely ill, showing dyspnea and moderate cyanosis. The temperature was 99° F., the pulse 120, and the respiratory rate 20. The blood pressure was 115/90 mm. Hg. The neck was short and thick and the great vessels were distended. The thorax was symmetrical, with equal expansion on both sides and no bulging of the intercostal spaces. The lung resonance was normal bilaterally. The heart was markedly enlarged but there was no evident pulsation in the precordial or great vessel areas. Sounds were distant and without murmurs. The liver extended three fingers' breadths below the costal margin and was smooth and tender. The spleen and superficial lymph nodes were not palpable. The skin over the entire body was moist and dusky, without eruptions or excoriations.

Laboratory Examinations: The urinalysis was essentially normal. The blood count showed: white blood cells 5150, 51 per cent segmented forms, 18 per cent band forms, 31 per cent lymphocytes; red blood corpuscles 3,900,000; and hemoglobin 70 per cent (Dare). A non-protein nitrogen determination was 46.5 mg. per cent. Roentgenological examination of the chest 3 days after admission showed some suggestion of fluid in the right pleural cavity with the heart enlarged to the left.

The patient's temperature ranged between 99° and 101° F. during his hospital course. His condition became progressively worse, with increasing dyspnea and cyanosis. His blood pressure was recorded as 120/0 mm. Hg. shortly before death on the 6th hospital day.

Postmortem Examination

The body was well nourished. There was cyanosis of the head and neck and slight generalized icterus. The skin was moist and showed no eruptions or gross lesions.

The pathological changes were confined to the heart and pericardium, pleura, diaphragm and liver. The general relationships of the thoracic viscera were distorted by the enormous enlargement of the heart and the presence of 2000 cc. of bloody fluid in the right pleural cavity. The right lung was partially collapsed.

The visceral and parietal pleurae showed numerous, densely adherent, soft hemorrhagic nodules which varied from 0.5 to 2 cm. in diameter. Similar nodules were present on the superior surface of the diaphragm. The left lung and pleural cavity showed nothing unusual. The mediastinal lymph nodes were markedly enlarged and hemorrhagic, and were incarcerated in a dense neoplastic mass which surrounded all the great vessels. The pericardium was thickened and the pericardial cavity obliterated by a similar neoplastic growth which varied from 2 to 3 cm. in thickness. The right auricle was markedly distended. Section revealed a tumor nodule originating in the wall and projecting into the auricular cavity. The endothelium was unbroken. The upper portion of the mass was continuous with the mediastinal lymph nodes. The nodule within the auricular cavity measured 6 cm. in diameter and practically occluded the tricuspid orifice. The valve leaflets were not involved. The right ventricle was collapsed; its wall was invaded to a moderate extent by the neoplasm from the pericardial cavity. The left auricle and ventricle both showed a similar neoplastic involvement. The pulmonary, mitral and aortic valves were unchanged though the aortic ring was definitely compressed by the surrounding tumor. The appearance of the neoplasm varied markedly in different portions. White mucoid areas alternated with large irregular blood sinuses, some of which were filled with bright red fluid blood while others showed areas of degeneration and were filled with a soft chocolate brown substance (Fig. 1).

The liver weighed 2070 gm. and showed advanced chronic passive congestion. On the superficial surface of the right lobe was a hemorrhagic nodule, 3 cm. in diameter, which somewhat resembled an area of infarction (Fig. 2). A similar nodule was noted in the quadrate lobe and two smaller ones in the central portion of the left lobe. In the cortex of each kidney were several, small, irregular hemorrhagic areas which also looked not unlike recent infarcts. The omentum was studded with small hemorrhagic nodules 2–3 mm. in diameter. Some of these were soft in consistence, others granular. Several similar nodules were present in the tissues surrounding the duodenum and jejunum. These did not communicate with the wall of the intestine. Examination of the entire gastro-intestinal tract revealed moderate congestion but no evidence of ulceration, neoplasia or obstruction.

Anatomical Diagnoses: Hemorrhagic neoplasm of the heart, with secondary nodules in the pericardium, mediastinum, pleura and liver; right hemothorax; hemorrhagic infarcts of the kidneys; and advanced passive congestion of the liver.

CASE 2. A white male, 30 years of age, a policeman, entered the hospital Dec. 26, 1937 complaining of weakness, shortness of breath, and tenderness in the upper right quadrant. He was born in the state of Oklahoma, but had resided in the city of Washington, D. C. for several years. The family history and previous history were non-contributory. In the early part of November he was in bed for a few days with a severe cold and bronchitis. On recovery he went back to work although he complained of being quite weak. On December 14th he returned to bed because of nausea and vomiting and tenderness in the upper right quadrant. His attending physician noted also a painless swelling of the patient's neck. The above symptoms subsided somewhat under bed rest but progressive dyspnea developed.

Physical Examination: On admission the physical examination revealed a well nourished man, acutely ill, with evident orthopnea and moderate cyanosis. The temperature was 99° F., the pulse 85, and the respiratory rate 20. The blood pressure was 110/0 in the right arm, 120/0 in the left arm. The neck was short, thick, and markedly congested. The thorax was of the hypersthenic type, symmetrical and with equal expansion on both sides. Lung resonance was good bilaterally. The heart was markedly enlarged both to the right and to the left. The point of maximum impulse was not palpable. The sounds were distant, weak and without murmurs. The abdomen was tender in the upper right quadrant and the liver palpable three fingers' breadths below the costal margin. The spleen and superficial lymph nodes were not palpable. The skin was moist and cyanotic. There were no lesions present.

Laboratory Examinations: Urinalysis was essentially normal. The blood count showed: white blood cells 13,300, 58 per cent segmented forms, 4 per cent band forms, 38 per cent lymphocytes; red blood corpuscles 4,300,000; hemoglobin 84 per cent (Dare). Roentgenological examination of the chest on December 31st revealed massive pericardial effusion.

Paracentesis of the pericardium was performed on December 31st, 720 cc. of bloody fluid being withdrawn. The fluid showed no growth on culture after 72 hours of incubation. The patient's dyspnea and cyanosis became more pronounced after the paracentesis and death occurred 2 hours later.

Postmortem Examination

The body was well nourished. The skin was cyanotic, especially in the region of the head and neck. The latter was short, stout, and appeared distended but not edematous. The superficial vessels of this region were markedly dilated and engorged. No cutaneous lesions of any sort were noted.

The essential pathological changes were confined to the heart, pericardial cavity and liver. The general relationships of the thoracic viscera were somewhat distorted. The pericardial sac

was distended by 1000 cc. of bloody fluid and the heart was displaced to the left. The right pleural cavity contained 1200 cc. of bloody fluid; the left 800 cc. Both lungs were partially collapsed and showed areas of atelectasis and emphysema, but no pneumonic, tuberculous or neoplastic involvement. The mediastinal lymph nodes were moderately enlarged but on section showed only anthracosis and congestion. The heart weighed 750 gm. The anterior surface consisted almost entirely of the right auricle, which measured 10 by 12 cm. in its oblique diameters. On opening the auricle a firm, compact tumor mass was found growing from the anterior wall into the auricular cavity which it practically obliterated. The tumor almost completely blocked the junction of the superior and inferior venae cavae and caused a ball valve type of obstruction at the tricuspid orifice. The endothelial lining of the auricle was smooth and unbroken (Fig. 3). On section through the tumor it was found to consist of a semisolid fleshy substance which varied in color and consistence in different portions. Centrally the tumor was soft, white and mucoid; at the periphery it was dense and reddish brown in color. A few hemorrhagic extravasations were noted in the upper portion, while on the auricular surface were present large blood sinuses, some containing bright red fluid blood and others a chocolate brown substance. There was no extension of the tumor into the left auricle or below the auriculoventricular junction. The right ventricle was partially collapsed, its wall moderately hypertrophied and toxic. The tricuspid and pulmonary valves showed nothing unusual. The left auricle and ventricle contained a small amount of clotted blood. Their walls were of normal thickness, color and consistence. The mitral valve was smooth and glistening. The aortic valve, while moderately compressed, was otherwise unchanged.

The walls of the various portions of the gastro-intestinal tract were thin. There was evidence of moderate passive congestion but no lymphoid hyperplasia or neoplastic involvement. The liver weighed 1850 gm. Externally and on cut section it showed marked chronic passive congestion with no gross or microscopic evidence of neoplasia.

Anatomical Diagnoses: Angioreticuloendothelioma (Kaposi's disease) of the heart; hemopericardium; bilateral hemothorax; and advanced passive congestion of the liver.

Microscopic Examination

Microscopic examination of hematoxylin-eosin stained sections from the primary tumors of the auricles in both cases and of the secondary nodules in the 1st case shows the neoplasms to be cytologically identical (Figs. 4 and 5). The disordered architectural arrangement and the multiplicity of cellular infiltrations and proliferations at once attract attention (Fig. 6). The structure in different portions is as variable as is the pleomorphism in individual regions. One obvious feature common to all sections is vascularity. In some areas the tumor is made up almost entirely of small, newly formed blood vessels with thin walls consisting only of endothelium resting on a reticular base. In other places large blood sinuses are present. These likewise possess thin walls which are frequently broken, permitting hemorrhagic extravasation. Some of the vessels are engorged with fresh blood while others are filled with degenerated débris and surrounded by massive irregular areas of liquefactive necrosis. Small areas of polymorphonuclear leukocytic and dense lymphocytic infiltration are dispersed throughout. The more solid portions of the tumor consist of proliferated embryonic spindle cells which vary in size, shape and arrangement. Some are small and closely packed together; others are large, almost oval, and possess hyperchromatic nuclei with an occasional mitotic figure (Fig. 7). Broad sheets of spindle cells invade the myocardium, resembling a rapidly growing fibrosarcoma (Fig. 8). In other places the cells appear more differentiated and assume a somewhat "windblown" appearance, or abruptly form irregular whorls suggestive of neurogenic origin (Fig. 9). Small areas of mucoid degeneration are found in the deeper portions of the tumor nodules.

The appearance of the predominating spindle cell suggests that it might have a fibroblastic origin, but sections stained by van Gieson's and Mallory's connective tissue stains show only the faintest trace of true connective tissue. Bielschowsky reticulum stains, however, show diffuse, massive reticular proliferation. In some areas the reticulum is fine, lace-like and compact (Fig. 10). In others, long thick processes are found which present a "broken twig" appearance similar to that seen in so-called Hodgkin's sarcoma (Fig. 11). The reticular arrangement around the blood

vessels is of interest. Each vascular space, whether of the capillary or the sinus type, is completely enveloped by reticulum which clearly outlines its wall (Fig. 12). In the areas of hemorrhagic extravasation a mosaic of fine reticular elements is attempting enclosure of the blood. In the myocardium and the liver, reticular proliferation precedes the infiltration of embryonic spindle cells. Sections stained by the Giemsa, the Gram-Weigert and the Ziehl-Neelsen methods reveal no bacteria, and Levaditi stained sections are negative for treponemas.

The tumors from the 2 cases are histologically identical with respect to both architectural arrangement and cytology and correspond in every way to the hemorrhagic sarcoma of Kaposi.

Morcellated portions of fresh tumor tissue from both cases and Berkefeld filtrates of such preparations were injected by various routes — subcutaneously, intraperitoneally, intravenously and intracerebrally — into a number of experimental animals including rabbits, mice, guinea pigs and canary birds. A localized pyogenic abscess developed in 1 rabbit at the site of the subcutaneous inoculation. With this exception no local or systemic reaction has been seen in any case up to the present time (151 and 178 days respectively since inoculation).

Discussion

The unusual features presented by the 2 cases herewith reported may be summarized as follows: (1) the comparative youth of the patients; (2) the fact that both of them were native-born Americans and one of them was an indoor worker; (3) the rapidly fatal course of the disease in both instances, which obviously was the result of primary cardiac involvement; (4) the unusual site of the primary lesion in the right auricle in both patients; and (5) the absence of skin lesions in both cases.

In our review of the literature of Kaposi's disease we were unable to find a single instance in which the condition was primary in the heart. Reviewing the literature on primary cardiac neoplasms, however, we did find 8 instances,[3, 6, 24, 27, 41, 52, 53, 57] in which the descriptions of the gross and microscopic appearance of the tumor strongly suggested that the conditions were similar to those in the cases herewith reported. Through the courtesy of Dr. T. B. Mallory we were able to examine the microscopic prepa-

ration from Case No. 22491,[6] which proved to be identical with our own cases. Thus it is evident that primary localization of Kaposi's disease in the heart is not as rare as would at first appear, the difficulty heretofore having been in its identification.

Of particular interest in the cases reported here is the fact that we were enabled to study the cytology of the lesion with especial thoroughness and to attempt transmission of the tumor to animals of many sorts and by various routes.

The results of these studies have given us a better insight into the nature and origin of these tumors. To the question, "Is Kaposi's disease an infectious granuloma?" it is our opinion that a negative answer should be given. With the single exception of Justus,[30] who in 1909 published a brief undocumented note on his animal experimentation, no student of the disease has been able to date to transmit it to lower animals. Pack [42] even attempted reinoculation of a patient with his own tumor without success. The results of our own particularly intensive attempts at transmission to animals are in line with these previous experiences. In summary, the work on this aspect of the disease may be said to suggest very strongly that the tumor is not a granuloma. Coupling this evidence with the grossly invasive character of the growth and the striking appearance of malignancy exhibited by microscopic preparations of the tumor, we have concluded that the disease is truly neoplastic. The "reticuloendothelial hyperplasia" theory seems as untenable as the "granuloma" theory because of the invasive character of the growth.

With respect to the specific type of neoplasm represented by the Kaposi tumor it becomes necessary to decide what tissue of the body could give rise to the numerous and diverse structures observed — newly formed blood vessels, including endothelium and adventitial connective tissue, embryonic spindle cells, lymphoid elements and well organized reticulum. Only from the reticuloendothelial system could all these types be derived. The tumor should therefore be given the name "reticuloendothelioma" to show this relationship. Since the neoformation of blood vessels is so outstanding a characteristic, it seems desirable to add the prefix "angio" to distinguish this tumor from other reticuloendothelial neoplasms in which other components predominate. As eponyms are at best undesirable makeshifts chiefly employed when

the true nature of a condition is obscure, and inasmuch as the disease Kaposi originally described was characteristically a skin disease, it seems desirable to use the nosological term "angioreticuloendothelioma" for this tumor at all times, save when the distinctive name "Kaposi's disease" may be applied specifically to the skin manifestations.

Summary and Conclusions

The problem of the origin and nature of the lesions of Kaposi's disease has been reanalyzed on the basis of a review of the literature on the subject and in the light of a thorough study of 2 recent cases in both of which the disease was primary in the right auricle of the heart and in which skin lesions were lacking.

It is concluded that the condition is a true neoplasm derived from the reticuloendothelial system with neoformation of blood vessels a prominent distinguishing characteristic.

Since tumors are properly named with respect to their tissues of origin, it is proposed that in the future the scientific term "angioreticuloendothelioma" be used in preference to the term "Kaposi's disease."

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DESCRIPTION OF PLATES

PLATE 30

FIG. 1. Case 1. Sagittal section of heart showing tumor filling right auricle and obliteration of pericardial space by neoplastic tissue.

FIG. 2. Case 1. Section of liver showing tumor nodule in right lobe.

FIG. 3. Case 2. Heart opened to show tumor mass filling right auricle. The unbroken endothelium of the auricle covers the nodule.

FIG. 4. Case 1. Microphotograph showing general character of the neoplasm, including proliferation of embryonic spindle cells and formation of new capillaries and blood sinuses. Hematoxylin-eosin stain. × 45.

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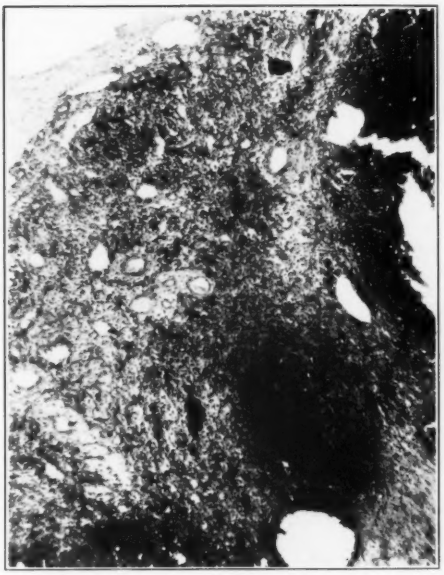

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PLATE 31

FIG. 5. Case 2. Microphotograph showing general character of neoplasm and illustrating particularly the similarity between the microscopic appearances of the two tumors. (See Fig. 4.) Hematoxylin-eosin stain. × 45.

FIG. 6. Case 1. Microphotograph showing multiplicity of cellular types. Small, thin-walled, newly formed blood vessels are seen below and a large blood sinus above. Hematoxylin-eosin stain. × 150.

FIG. 7. Case 2. Microphotograph showing cytological characteristics of the infiltrating embryonic spindle cells. Heart muscle fibers are seen at the right below. Hematoxylin-eosin stain. × 300.

FIG. 8. Case 2. Microphotograph showing invasion of myocardium by tumor tissue. Hematoxylin-eosin stain. × 175.

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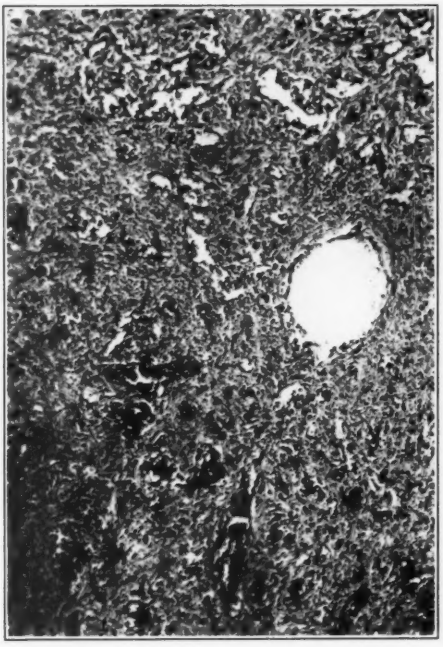

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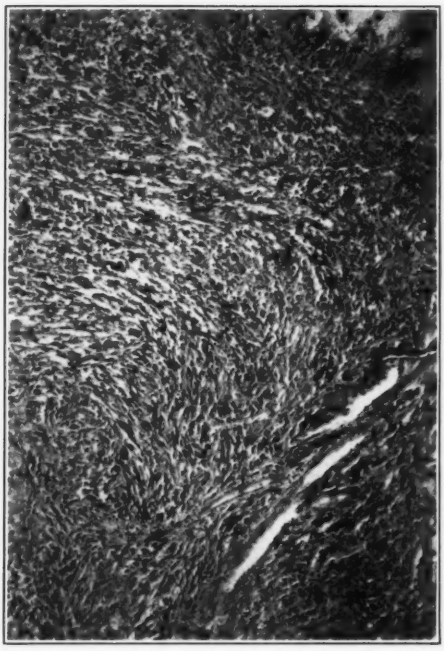
Plate 32

Fig. 9. Case 2. Microphotograph of an area in which the spindle cell component of the tumor has a "windblown" appearance suggestive of neurogenic origin. Hematoxylin-eosin stain. × 150.

Fig. 10. Case 1. Microphotograph of a section stained by the Bielschowsky method. There has been massive proliferation of fine, compact, lace-like reticulum. × 300.

Fig. 11. Case 2. Microphotograph showing "broken twig" type of reticulum proliferation. Bielschowsky's silver impregnation method. × 300.

Fig. 12. Case 1. Microphotograph showing structure of the reticulum in a vascular portion of the tumor. Each vascular space is enveloped by reticulum which clearly outlines its wall. Bielschowsky's silver impregnation method. × 400.



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THE PATHOLOGY OF NUTRITIONAL MUSCULAR DYSTROPHY IN YOUNG RATS *

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The young of female rats fed certain restricted vitamin E diets develop nutritional muscular dystrophy. The paralytic symptoms of this disease were observed for the first time in the laboratory of Evans and Burr,[1] and the pathology was described by Olcott.[2] Symptoms of weakness of the muscles have been reported in older animals maintained on vitamin E-low diets from the time of weaning (Ringsted,[3] Burr, Brown and Moseley,[4] Einarson and Ringsted,[5] Evans, Emerson and Telford,[6] and Knowlton and Hines [7]). Several of these authors have also described early lesions in the muscles.

The disorder is prevented by the administration of wheat germ oil or vitamin E concentrates to either the mother or her young during the period of lactation (Evans and Burr,[1] Ringsted,[3] Morelle,[8] Mason,[9] and Olcott and Mattill [10]). However, when natural food diets in which the antisterility factor has been inactivated by treatment with ethereal ferric chloride (Waddell and Steenbock [11]) are fed, the young rats remain normal (Goettsch and Pappenheimer [12]). This discrepancy has been substantiated recently by the report of Goettsch and Ritzmann [13] that young rats are protected against disease of the muscles by supplementing the diet with wheat germ oil, with oil of treated wheat germ in which the antisterility vitamin has been inactivated by ethereal ferric chloride, or with α-tocopherol (vitamin E).

This paper concerns the pathological changes in the young rats studied by Goettsch and Ritzmann.[13] Our observations confirm those of Olcott [2] in all essentials. The alterations are strictly limited to the voluntary muscles. Although the central nervous system and peripheral nerves were not studied in every animal, a searching examination was made in several typically affected

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rats.* No lesions were found in the brain, spinal cord or peripheral nerves, and even the terminal neurites and end plates were well preserved in the midst of the degenerated muscle fibers (Fig. 1). These findings are in accord with those of Barrie [14] and of Olcott,[2] who found no abnormalities in the nervous system, but are opposed to those of Lipschutz,[15] who described degeneration of crossed and homolateral vestibular tracts, and of the posterior tracts of Goll and Burdach, the median lemniscus, and the tectospinal tract. Neither Barrie nor Lipschutz appears to have studied the skeletal muscles.

We have also been unable to confirm the observations of Barrie on the cytological changes in the anterior lobe of the pituitary. In the glands studied by us we failed to note the degranulation of eosinophils and the spongy appearance of the basophils, which this author described as characteristic. The thyroid also showed no striking difference from that of normal rats, an observation in accord with that of Telford, Emerson and Evans.[16] The thymus was well preserved but in one animal showed a moderate accidental involution, which can probably be ascribed to starvation rather than to a specific effect of the diet. The remaining glands of internal secretion, as well as the principal viscera, showed no significant alterations. The myocardium, the smooth muscle of the intestine and other tissues were not affected.

The gross appearance of the muscles varied according to the number of days elapsing after the onset of symptoms. In rats sacrificed on the day when weakness of the muscles first became apparent the muscles appeared moist and somewhat pale. After 2 days the muscles became yellowish white and opaque. After 3 days or more the muscles appeared streaked and gritty. The muscles of both upper and lower extremities, as well as those of the trunk, were involved but not necessarily symmetrically, nor were the individual muscles always affected in their entirety. Often a band of intact non-degenerated fibers persisted on the surface. As in the muscular dystrophy of guinea pigs (Goettsch and Pappenheimer [12]), the muscles of the tongue seemed to be unaffected. The masseter was found to be involved in several cases.

* We are greatly indebted to Dr. Abner Wolf for his collaboration in the study of the nervous system.

The microscopic changes also depended on the interval between the onset of symptoms and the time that the animals were sacrificed. The earliest change was a necrosis of the fibers, with retraction and separation of the muscle substances into discrete segments and collapse of the intervening sarcolemma (Fig. 2). In some instances the muscle substance underwent complete hyaline necrosis with loss of all fibrillar structure, but at times the longitudinal striations could still be discerned in the necrotic fibers and persisted even after calcium impregnation had occurred.

Accompanying this alteration in the muscle fibers there was noted on the 1st day a marked interstitial edema, in part fibrinous, which led to wide separation of the fibers (Fig. 3). There was also a marked inflammatory reaction, in the early stages of which polymorphonuclear leukocytes predominated. Occasionally these penetrated the necrotic fibers, but in general they remained confined to the interstitial tissue. On the 1st day there was little alteration of the nuclei of the sarcolemma.

In the animals killed on the 2nd day the edema had largely been resorbed and polymorphonuclears had given way to mononuclear histiocytes. The myocytes were becoming activated, their nuclei were vesicular, and their cytoplasm had increased in volume and was basophilic. Mitotic figures were quite numerous (Fig. 4). The segments of necrotic muscle were becoming enveloped in plasmatic multinucleated cell masses, probably derived from the persisting myocytes, although the participation of histiocytes in this process could not be excluded (Fig. 5). In some instances calcification of the necrotic fibers had begun, although this was more conspicuous and was seen more frequently on the 3rd and subsequent days (Fig. 6).

The specimens from the rats killed on the 3rd and 4th days showed in addition marked regenerative activity on the part of the myocytes. Many of them had taken on spindle or cylindrical shapes, and myofibrils were becoming differentiated on their surfaces. With their development into functional muscle cells the staining again became eosinophilic, presumably due to the regeneration of myohemoglobin. In later stages they could still be distinguished from the persistent unaffected fibers by their smaller caliber, by the central location of their nuclei, and by a decrease in myofibrils. In a specimen (Rat 21 c) obtained on

the 3rd day (Fig. 7) the necrotic fibers had been completely resorbed and their place was taken by well differentiated young fibers aligned in the original axis of the muscle.

We have not as yet examined rats at a later stage of recovery but it seems probable that the regeneration is complete in time and that no permanent scarring of the muscle persists.

Discussion and Conclusions

Our material does not permit any positive conclusions as to the pathogenesis of these lesions. One may conceive of such changes as brought about (*a*) by excessive contraction of fibers with segmental ruptures and subsequent necrosis, (*b*) by a direct and selective toxic action on the muscle cells, and (*c*) by angiospastic occlusion, causing anoxemia and infarction. Although speculation is hardly warranted, the fact that the superficial fibers of a muscle, which perhaps receive a more direct and abundant blood supply from the intermuscular fascia, often escape necrosis (Fig. 8) may perhaps be taken as a point in favor of the last hypothesis. We made a careful examination for capillary thrombi in the early stages of the disease but they were not found. However, the capillaries of the muscles are strikingly empty and collapsed when contrasted with the normal muscle tissue.

Whatever the pathogenesis, the degeneration of the fibers seems to occur with almost explosive suddenness. Later stages are those of reaction to the necrotic tissue and of early and active regeneration. It is thus possible to estimate the duration of the symptoms with reasonable accuracy and to predict that survival will be accompanied by practically complete restoration to normal structure.

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DESCRIPTION OF PLATES

PLATE 33

FIG. 1. Rat G 15. Section showing the preservation of neurites and motor end plates in degenerated muscle. Modification of Cajal's method. × 700.

FIG. 2. Rat G 51 a. Killed on day after appearance of symptoms. Segmental necrosis of muscle fibers, interstitial edema and leukocytic reaction is present. Hematoxylin-eosin stain. × 170.

FIG. 3. Rat G 51 a. Showing a higher power of the same section. × 700.

FIG. 4. Rat G 21 e. Killed on the 25th day, 3 days after the appearance of symptoms. Marked proliferation of myocytes with numerous mitoses is seen. Hematoxylin-eosin stain. × 700.

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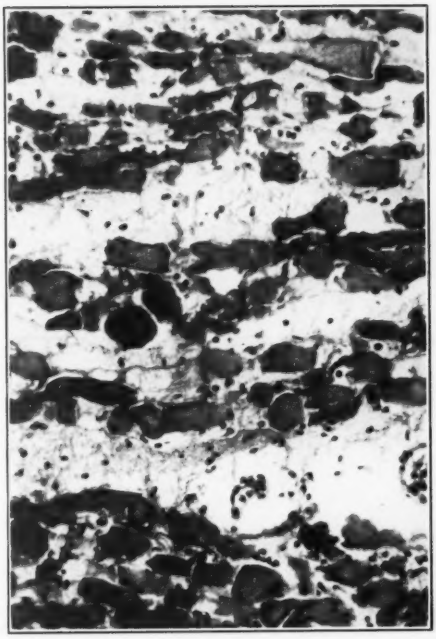

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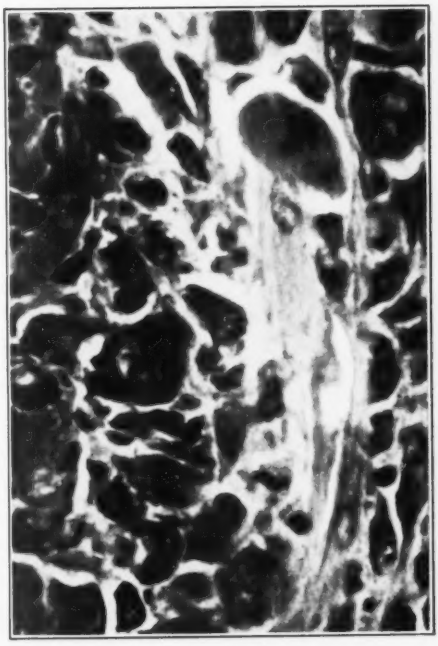

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Pappenheimer

Nutritional Muscular Dystrophy in Rats

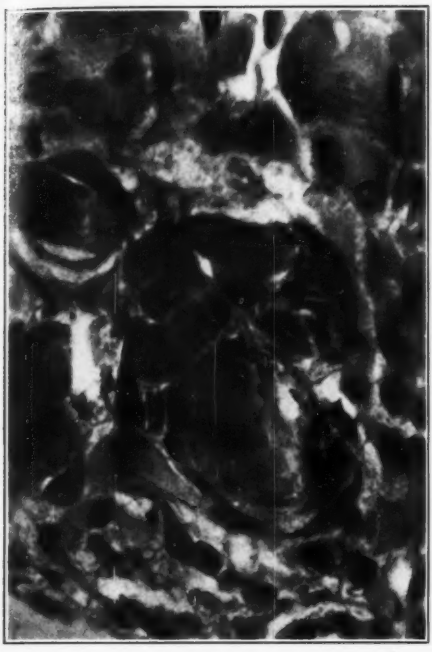
PLATE 34

FIG. 5. Rat G 60 b. Killed on 25th day, 3 days after symptoms were first noted. Necrotic, partly calcified muscle substance surrounded by multinucleated plasmatic masses is seen. Hematoxylin-eosin stain. × 700.

FIG. 6. Rat G 21 d. Killed on 25th day, 3 days after appearance of symptoms. Extensive calcification of necrotic fibers is present. Von Kossa's stain. × 170.

FIG. 7. Rat G 21 c. Killed on 25th day, 3 days after appearance of symptoms. Regenerated fibers of small caliber and centrally disposed nuclei, contrasting with normal unaffected fibers, is seen. Late stage in regeneration. Hematoxylin-eosin stain. × 170.

FIG. 8. Rat G 21 e. Preservation of superficial portion of muscle with advanced degeneration of the central portion is seen. Hematoxylin-eosin stain. × 170.



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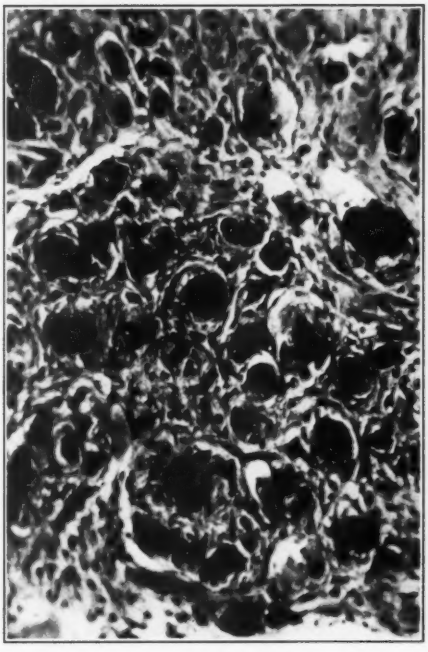

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Pappenheimer

Nutritional Muscular Dystrophy in Rats

THE INFLUENCE OF INFLAMMATION ON THE SKIN NECROTIZING ACTION OF STAPHYLOCOCCUS TOXIN *

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Although it has been established that under certain conditions an inflammatory reaction may function in producing a non-specific local cutaneous immunity, the basic mechanism involved in such immunity continues to be a subject of opposing hypotheses and divergent opinions. Much has been written concerning the fixation or localization of various irritants in foci of inflammation and the protection afforded the organism as a whole through this reaction. The opposite idea, however, of local protection due to an accelerated removal or dissemination of the irritant has not been adequately investigated.

The findings presented in this communication demonstrate the capacity of a mild cutaneous inflammation to confer protection against the injurious local effects of the toxin of *Staphylococcus aureus*.

Previous studies on the role of inflammation in immunity have dealt with the fate of such substances as dyes, bacteria and foreign proteins following their injection into inflamed tissues.

Menkin [1] demonstrated that when trypan blue was injected directly into an area of inflammation prepared by the subcutaneous application of aleuronat, the dye was not found in either the efferent lymphatics or the regional lymph nodes. The author explains this on the basis of thrombosis of lymphatic vessels and a rapid "walling-off" of the inflamed area. Miller [2] found that inflammation inhibited the absorption of slowly diffusible compounds and increased the dissemination of highly diffusible substances.

The results obtained by Okuneff,[3] on the other hand, indicate that local inflammation may facilitate the dissemination of a colloidal dye. He showed that if a small amount of turpentine was injected into the subcutaneous tissues immediately preceding the injection of trypan blue, a marked acceleration in the rate of absorption of the dye into the blood stream occurred. If, however,

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the dye was introduced 12 hours after the turpentine the rate of absorption of the dye was greatly reduced.

Hudack and McMaster [4] injected dyes into skin sites inflamed by heat, ultraviolet light or bacterial toxins and noted that dye dissemination, as judged by skin decolorization, was greatly accelerated in the inflamed areas. Here the inflammatory reactions were induced 24 hours before instillation of the test dye solution and yet there was no indication of any process of "fixation" or lymphatic thrombosis.

The findings given by Falchetti and Carlinfanti [5] concerning the increased sensitization to anaphylaxis following injection of a foreign protein into a focus of inflammation are probably to be explained on the basis of greater absorption of the antigen from an inflammatory reaction site.

The inadequacy of allergic inflammation as a localizing mechanism is clearly shown in the work of Cannon and Hartley.[6] When they introduced virulent pneumococci directly into sites giving the Arthus reaction the organisms were not localized at the point of inoculation. Animals so treated succumbed to the infection just as quickly as did those in which the microörganisms were injected into normal skin. In some cases the inflammation seemed to favor the growth and dissemination of the bacteria.

Materials and Methods

The toxin was prepared from stock cultures of *Staphylococcus aureus*, several different strains being employed. Proteose-peptone semisolid medium was dispensed in 100 cc. amounts into 500 cc. Ehrlenmeyer flasks and autoclaved at 15 pounds for 15 minutes. The flasks were inoculated with 8 hour broth cultures, placed in a desiccator with an atmosphere of 20 per cent carbon dioxide and 80 per cent air and incubated at 37° C. for 3 to 4 days. The culture was then filtered through paper, adjusted to a pH of 6.6 and further cleared by centrifugation. The toxic filtrate was preserved by the addition of merthiolate to a concentration of 1:10,000 and was then stored in the refrigerator.

Potent immune serum against sheep serum and against crystalline egg albumin was obtained by injecting rabbits with multiple doses of the corresponding antigen. Injections were continued until the antiserum reached a titer of 1:20,000 to 1:30,000 when

tested against the homologous antigen by the precipitation method.

Healthy young rabbits weighing from 1800 to 2200 gm. were used in these experiments. They were clipped and shaved 2 days before the test and no animals were used a second time.

The Influence of Allergic Inflammation on the Necrotizing Action of Staphylococcus Toxin

In order to determine the effect of allergic inflammation on the ability of the toxin to produce local necrosis of skin, the following procedure was employed.

Fresh, undiluted immune rabbit serum was infiltrated into the prepared skin of a normal rabbit for the purpose of local passive sensitization. Eight to 10 intracutaneous injections of 0.1 cc. each were made in an area of skin about 25 mm. in diameter. Five to 7 hours later the animal was given, intravenously, a dose of antigen specific for the immune serum used, and 0.1 cc. of diluted *Staphylococcus* toxin was injected into the skin of the infiltrated area. Suitable controls were set up for each of the reagents used and daily observations were made as to the character and size of the resulting lesions.

For the sake of convenience the tests were run in three series of 10 animals each. In 6 animals of each series antiegg albumin serum was injected into one flank and antisheep serum in the opposite flank as a further check on the inhibiting effect of the immune serum alone. Table I is a typical protocol showing the results obtained in one such series.

Inasmuch as the area of skin necrosis had usually reached its limit by the end of 48 hours, the dimensions of the necrotic lesion and the number of days required for healing are taken as important points of contrast. As may be seen from Table I, these two observations alone would serve to demonstrate that a mild inflammatory reaction may function to protect a local area of skin against the effects of the toxin. However, the differences in the *character* of the lesions are equally important. The necrotic lesions in the tissues which were the site of allergic inflammation were not only smaller but were also much less severe than lesions in the control areas. In the inflamed areas the necrosis involved only the superficial layers of skin and the brown necrotic crusts that formed after several days were quite thin, while in the control

sites the necrosis often extended to the subcutaneous fascia and the crusts were thick and "leathery."

The Protective Action of Serum Alone

In all of the experiments discussed in the previous section fresh serums, taken less than an hour before use, were employed

Table I

Effect of Allergic Inflammation on Skin Lesions Produced by Staphylococcus Toxin

Animal No.	Intradermal injections	Interval	Intravenous antigen	Toxin	Size of lesion *	Lesion healed
	serum	*hrs.*		*cc.*	*mm.*	*days*
17	*Right*: none *Left*: antiegg albumin	6	Egg albumin	0.1 0.1	20 × 50 5 × 8	33 14
57	*Right*: normal rabbit *Left*: antiegg albumin	5	Egg albumin	0.1 0.1	25 × 35 4 × 7	30 12
26	*Right*: none *Left*: antiegg albumin	5	None	0.1 0.1	30 × 30 25 × 35	32 30
56	*Right*: none (saline) *Left*: antisheep	7	Sheep serum	0.1 0.1	20 × 40 8 × 8	31 15
70	*Right*: antiegg albumin *Left*: antisheep	6	Sheep serum	0.1 0.1	20 × 30 5 × 5	33 13
86	*Right*: antiegg albumin *Left*: antisheep	6	Sheep serum	0.1 0.1	30 × 35 5 × 8	34 12
76	*Right*: antisheep *Left*: antiegg albumin	5	Egg albumin	0.1 0.1	25 × 40 9 × 9	29 15
66	*Right*: antisheep *Left*: antiegg albumin	7	Egg albumin	0.1 0.1	20 × 30 5 × 5	30 14
85	*Right*: antisheep *Left*: antiegg albumin	6	Egg albumin	0.1 0.1	30 × 40 8 × 8	32 15
36	*Right*: antisheep *Left*: antiegg albumin	6	Egg albumin	0.1 0.1	25 × 35 4 × 9	31 13

* Taken 48 hours after injection of toxin.

for infiltration of skin areas. Such serums alone rarely caused any inhibition of the toxic action.

It was noted, however, in the course of this work that injections of old serums that had stood in the refrigerator for several days or more would confer protection against the toxin without antigenic participation. Within 5 or 6 hours after cutaneous infiltration with these old serums the site was erythematous and somewhat indurated, and lesions resulting from the injection of *Stapyhlococcus* toxin into these areas were no more extensive than those in the sites of allergic inflammation. Passage through a Berkefeld filter did not remove the irritating property from these serums; the filtered serums alone would still confer protection against the morbid effects of the toxin.

Discussion

It is quite evident from these experimental procedures that *Staphylococcus* toxin fails to exert the full force of its typical necrotizing property in the presence of a mild inflammatory reaction. Complete protection is not afforded by this means but marked inhibition of the toxic action is observed with striking regularity.

An important fact with regard to those experiments in which the meeting of antigen and antibody within the tissues was employed as the inflammatory irritant is that the toxin was actually introduced before inflammation was accomplished. Allergic inflammation did not develop, of course, until sometime after the intravenous administration of the homologous antigen. In those instances of infiltration of the skin with aged serum, a mild inflammatory reaction of some 5 hours duration was present at the time the toxin was injected. In either case, however, there resulted a striking diminution in the display of toxic action.

A further point to be considered is that the injection of *Staphylococcus* toxin into the skin is not comparable to the instillation of dyes or foreign proteins or even of virulent staphylococci. We have, in the case of the toxic filtrate, a substance which in itself is capable of setting up severe inflammation with necrosis of tissue. In spite of this the inflammatory and necrotizing potencies of the toxin are greatly lessened under the conditions of the present investigation. Instead of there being an additive action

of the two processes or of the irritating properties of the toxin being superimposed on the allergic inflammation, we observe a retardation or partial loss of the inflammatory propensities of the toxin.

In considering the protective mechanism responsible for the phenomena observed in the present study, the concept of fixation or localization [7] of the toxin does not appear to be tenable here. There is, of course, spreading of the toxin following its injection into normal skin, whereas when this irritant is introduced into inflamed cutaneous tissue the resulting necrosis is confined to a small area immediately around the point of injection. However, if the total amount of the injected toxin is merely fixed or localized in a smaller area, one would expect to see a more intense or more severe reaction than where the whole effect is spread over a larger area. The lesion in the inflamed skin is not only confined to a smaller area, but it is actually less severe and usually heals in half the time of the large lesions.

Likewise, due to the character of the lesions, any explanation based on the idea of inflammation causing a reduction in tissue permeability [8] or inhibition of the spreading factor found in certain toxic filtrates [9] would not seem to be applicable here.

It is a well established fact that in inflammation there are certain changes in the endothelial lining of the blood capillaries which increase their permeability or capacity for passing fluids. One finds in the work of McMaster and Hudack [10] and Hudack and McMaster [4] evidence that the permeability of the lymphatic capillaries is also altered so that they become more permeable under the influence of inflammation. Other workers [11, 12] have determined that there is actually an increase in the flow of lymph from a region of inflammation. Drinker and Field [12] conclude that "all evidence points to the fact that the more permeable the lymphatic capillary the better it must function in tissue fluid drainage."

In view of the fact that there is an increase in capillary permeability and a greater turnover of fluid in inflamed tissues, it would appear that the findings of the present investigation could be adequately accounted for on the basis of dilution and rapid removal of the irritant. The mechanism of the protective effect of inflammation under the conditions of the present study is prob-

ably a phenomenon of accelerated dissemination of the toxin from the site of injection into the systemic circulation.

It is not to be inferred from this discussion that the function of inflammation with reference to immunity is similar under different conditions. The character and intensity of the inflammatory reaction and the nature of the irritant must all be taken into consideration in evaluating the protective role. In severe inflammatory processes microörganisms are localized [13] and the vital organs may be protected at the expense of local injury. But, on the other hand, the diffusion of obnoxious substances from a focus of relatively mild inflammation may be facilitated and thus the organism as a whole be threatened.

Summary and Conclusions

Rabbits were used to study the effect of an early inflammatory process on the characteristic skin necrotizing action of *Staphylococcus* toxin. Local cutaneous areas were passively sensitized by the intradermal infiltration of immune serum. A few hours later toxin was injected into the infiltrated site and an allergic inflammation was induced by the intravenous administration of homologous antigen. It was demonstrated that the effect of the toxin was markedly inhibited by the inflammatory reaction, and the necrotic lesions were smaller, less severe and healed much more quickly than the controls.

The significance of these observations as regards accelerated dissemination of the toxin has been discussed.

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DESCRIPTION OF PLATE

PLATE 35

FIGS. 1 and 2. Rabbit 17. Lesions produced by *Staphylococcus* toxin in normal skin (Fig. 1) and in an area previously infiltrated with antiegg albumin serum (Fig. 2) 48 hours after the injection of 0.1 cc. of toxin into each area and the intravenous injection of crystalline egg albumin.

FIG. 3. Rabbit 70. Necrotic lesion resulting from the injection of 0.1 cc. of the toxin into skin previously infiltrated with antiegg albumin serum.

FIG. 4. Showing the extent of necrosis in the same animal after injection of 0.1 cc. of toxin into an antisheep serum infiltrated area and the intravenous administration of sheep serum. The small areas of discoloration around the central lesion are due to the fading allergic reaction. Both photographs were taken 48 hours after the injection of toxin.

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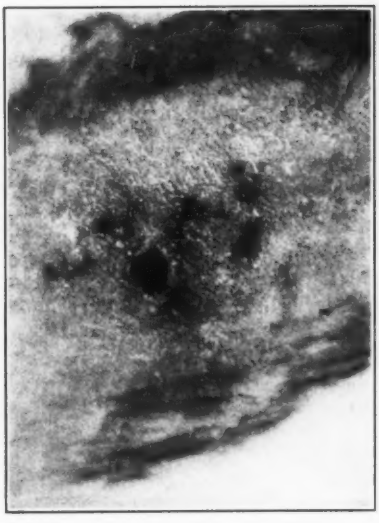

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Kenton

Necrotizing Action of Staphylococcus Toxin

REPAIR IN EXPERIMENTAL PNEUMOCOCCIC MENINGITIS

A Histopathological Study of Residual Lesions in Rats *

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The nature of the residual lesions that follow recovery from purulent meningitis has rarely been described. The only study found in the literature is that by Winkelman and Eckel,[1] which described such lesions in a 10 year old girl who died of an accident 2 weeks after recovery from acute meningococcic meningitis. In this case the pia was reported to be almost entirely free of inflammatory elements, although a relatively slight but general increase in connective tissue was present. The most striking finding was the presence in the cortex of acellular areas (Verödungsherde) of various sizes, most of them small and perivascular. Slight hyalinization and thickening of cortical and subcortical vessels associated with perivascular edema were noted and, at times, pigment-laden phagocytic cells were seen in the Virchow-Robin spaces. A 2nd case with similar findings was mentioned by the same authors.

Although it has been reported that a few experimental animals have recovered from pneumococcic meningitis,[2, 3] no study of residual lesions has been published. Opportunity for such an investigation arose during the course of our various chemotherapeutic experiments on white rats in which pneumococcic meningitis (types I and II) was produced.[4, 5] A significant number of the treated animals recovered and 59 of them were sacrificed 3 to 6 weeks following infection in order to study the brains and spinal cords.

All animals were killed with ether and skinned, the parietal plates removed, and the vertebral column with attached skull was placed in 10 per cent formalin. After fixation the brain and cord were carefully removed so as not to strip the pia. In later experiments considerable time was saved by removing the brain with cord prior to fixation. In most instances the brain was divided

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into five to six coronal sections, and the cord into six to seven cross sections. The tissues were stained with hematoxylin and eosin and by the Nissl method.

Gross examination did not reveal lesions of the brains or cords. Microscopically, however, the pia of every brain and of about one-third of the spinal cords showed pathological changes. The parenchyma of the brain exhibited focal cortical lesions of different varieties in over one-half of the animals.

Meningeal Lesions

Evidence of a healed or of an active chronic inflammation was present in the leptomeninges of every animal. The lesions, exudative and proliferative in character, were commonly focal, predominantly involved the free surface of the cerebrum and the pons, and were infrequently observed in the cerebral fissures or over the cerebellum. Cellular infiltrations were frequently perivascular, particularly about larger vessels (Fig. 1), but were also found diffusely distributed over limited areas. In addition to lymphocytes and histiocytes there were mononucleated cells with abundant basophilic cytoplasm and central round nuclei, the chromatin of which did not possess the arrangement characteristic of plasma cells. Occasionally, large mononucleated phagocytic cells containing hemosiderin were seen.

A not uncommon change, more or less generalized, was a moderately intense basophilia of the cerebral and cerebellar pia associated with a slight increase in thickness of the membrane and excessive prominence of pial nuclei. A similar prominence of nuclei was observed in many of the smaller meningeal vessels. More frequently, foci of fibrous tissue proliferation were encountered. This connective tissue was usually arranged in a loose meshwork of fibrils throughout which the inflammatory cells were disposed. Occasionally the collagenous fibers formed a thick, wavy, more compact membrane (Fig. 2).

Hemosiderin, usually intracellular, was present in a few foci commonly associated with larger deposits in the superficial cortex. Similarly, calcification of the pia was secondary to larger calcific deposits in the subjacent cortex (Fig. 3).

The meninges of the spinal cords were involved in a manner similar to those of the brain, but less frequently and to a much

lesser degree. The changes were strictly focal and the foci were, as a rule, quite small. The exudation, almost entirely lymphocytic in character, was scanty and the pial fibrosis was slight (Fig. 4). In a number of animals spinal ganglia and nerve roots exhibited peripheral lymphocytic infiltration, and in one instance numerous eosinophils participated. No hemosiderin deposits were encountered although several small foci of calcification were found involving both the pia and the dura.

Brain Lesions

The brain substance exhibited more striking lesions which, however, were present in only one-half of the animals and were strictly focal and frequently solitary. In some animals extensive shallow cortical scars were found, whereas in others the lesions were deeper and conical in shape with the base toward the surface (Fig. 5). These scars were exceedingly vascular and contained, in addition to many small round cells, larger glial cells and numerous hemosiderin-filled gitter cells. The vessels, most of them of the capillary type, were greatly increased in number and often appeared to spread fan-like from the inferior tip of the wedge shaped scar. They appeared quite prominent, due to the presence of swollen, deeply staining lining endothelial cells and to clusters of perivascular macrophages.

Some of the cortical scars showed calcium deposits in the form of large cohesive masses (Fig. 3), of more loosely dispersed granules (Fig. 6), or of small spicules. In several animals there was a large, subpial ovoid mass of fairly dense collagen in the center of a cortical scar. A concentric arrangement of oval and spindle shaped nuclei contributed a whorl-like appearance to the collagenous mass (Fig. 7). In addition, these masses contained granules of calcium salts and of hemosiderin.

In 2 animals extensive cortical destruction with associated complete disappearance of nerve cells and subsequent scar formation resulted in a marked localized dilatation of the lateral ventricle in that region (Fig. 8).

Cerebellar involvement, consisting of focal atrophy and complete absence of the molecular layer, was found in a few rats. In these regions the pia was slightly thickened and separated from the granular layer only by loosely arranged fibrils, a few lympho-

cytes, and clusters of hemosiderin-filled phagocytes. The granular layer in this region showed a striking absence of Purkinje cells.

In the paraventricular regions many of the animals showed gitter cells containing hemosiderin. This pigment was also present extracellularly.

The focal rarefaction of brain tissue described for the acute stage of purulent meningitis was not found except in and about cortical scars. Such rarefaction with associated disappearance of neurones is well illustrated in Figure 8. At an early stage in this study focal rarefaction of the cerebral cortex was thought to be frequent, but a more careful scrutiny of these foci demonstrated them to be a part of the architectural pattern normal for those particular regions.

Discussion

The data obtained in the present study indicate that in experimental pneumococcic meningitis resolution of the exudate usually occurs to a degree comparable to that seen in experimental pneumococcic pneumonia of rats.[6, 7] Similar to the interstitial inflammation and foci of organization seen following recovery from pneumonia,[6, 7] foci of residual inflammation may be found following recovery from pneumococcic meningitis. These foci, however, are relatively few in number and often solitary. It is not unlikely that such solitary lesions might be difficult to find and could easily be missed in the brains of larger animals. Nevertheless, strategically situated in a patient they could conceivably give rise to a variety of nervous manifestations. The increasing incidence of recovery in clinical purulent meningitis [4, 8] may afford opportunities for verifying this conjecture.

The lesions herein described were predominantly of a productive inflammatory type and differed thereby from the essentially degenerative lesions described by Winkelman and Eckel.[1]

The proliferation of vascular adventitial macrophages, which played a prominent part in the structure of cortical scars, was similar to the clasmatocytic proliferation described by Kubie [9] in experimental herpetic encephalitis, and later by Stewart [10] in experimental pneumococcic meningitis of dogs.

Finer histological changes, hyalinization with thickening of vessels and perivascular edema were not noted.

Summary

In a study of the central nervous system of 59 rats that had recovered from pneumococcic meningitis, exudative or proliferative meningeal changes were found in every animal. Lesions of the brain parenchyma, consisting of scars, cellular infiltration and calcium or hemosiderin deposits, were found in about one-half of the rats, while only about one-third exhibited lesions in the meninges of the spinal cord. The spinal meningeal changes were usually slight and were not associated with parenchymatous lesions.

Note: The authors wish to thank Dr. H. T. Karsner and Dr. Harry Goldblatt for their helpful criticism.

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DESCRIPTION OF PLATES

PLATE 36

FIG. 1. Lymphocytic infiltration of the pia arachnoid, predominantly perivascular in character. Type I pneumococcic meningitis. Treatment with sulfanilamide and serum. Rat sacrificed 34 days following infection. Hematoxylin-eosin stain. × 114.

FIG. 2. Fibrosis of the pia arachnoid with associated cellular infiltration. Type II pneumococcic meningitis. Treatment with sulfanilamide. Rat sacrificed 22 days following infection. Hematoxylin-eosin stain. × 100.

FIG. 3. Shallow cortical scar with large cohesive mass of calcific material involving the pia also. Prominent, deeply staining capillaries are seen beneath the mass. An area of rarefaction with clusters of hemosiderin-filled gitter cells is present at a lower level. Type II pneumococcic meningitis. Treatment with sulfanilamide. Rat sacrificed 29 days following infection. Hematoxylin-eosin stain. × 150.

FIG. 4. Lymphocytic infiltration of the spinal meninges. Type I pneumococcic meningitis. Treated with sulfanilamide and serum. Rat sacrificed 34 days following infection. Hematoxylin-eosin stain. × 122.

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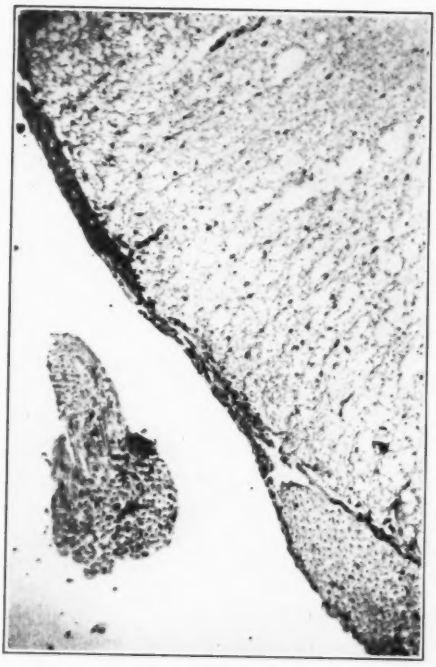

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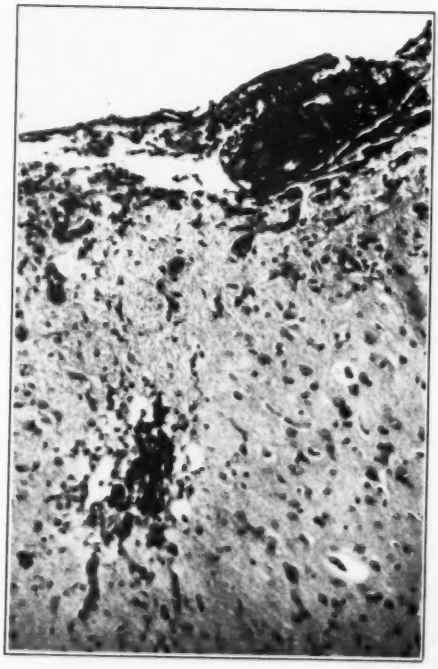

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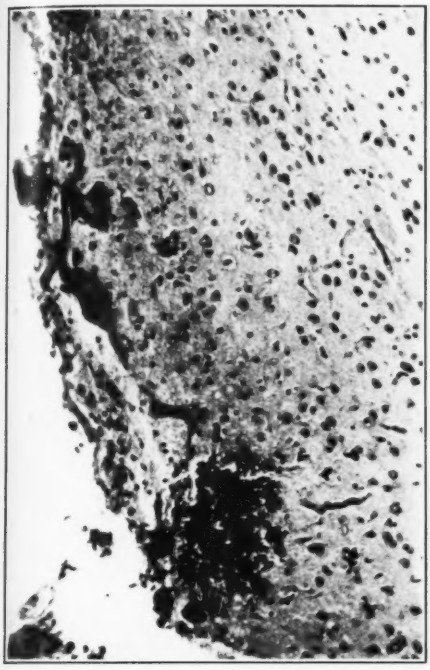
PLATE 37

FIG. 5. Cellular cortical scar containing many hemosiderin-filled phagocytes. Same animal as in Figure 2. Hematoxylin-eosin stain. × 122.

FIG. 6. Cortical scar containing calcium in granular dispersed form. A number of prominent, deeply staining vessels are present. Type I pneumococcic meningitis. Treated with sulfanilamide and serum. Rat sacrificed 38 days following infection. Hematoxylin-eosin stain. × 150.

FIG. 7. Cortical scar containing a large collagenous whorl. The large, deeply staining cells are hemosiderin-filled phagocytes. Same animal as in Figures 2 and 6. Hematoxylin-eosin stain. × 70.

FIG. 8. Extensive shallow cortical scar with rarefaction, absence of neurocytes and atrophy. The concave edge represents the attenuated ependyma of the dilated lateral ventricle. The conspicuous character and large number of the vessels is well illustrated. Type I pneumococcic meningitis. Treatment with sulfanilamide and serum. Rat sacrificed 37 days following infection. Hematoxylin-eosin stain. × 110.



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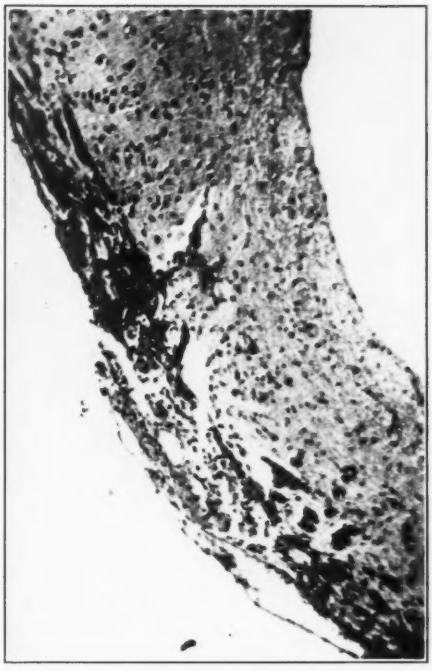

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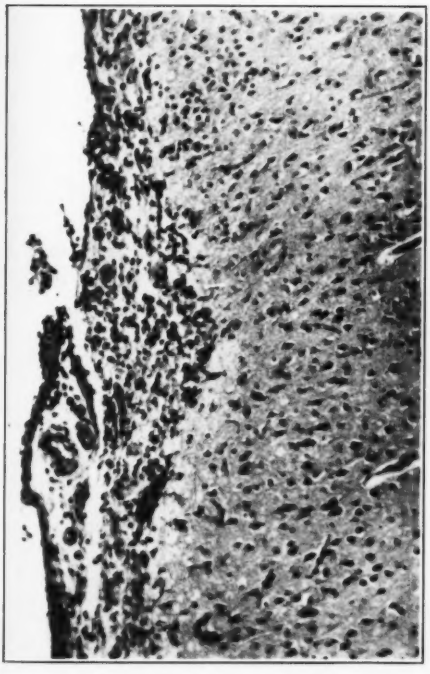

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THE EFFECT OF DIET ON THE PATHOLOGICAL CHANGES IN RATS WITH NEPHROTOXIC NEPHRITIS *

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Nephrotoxic nephritis has been regarded by Masugi and the majority of recent workers as the experimental renal lesion which most closely simulates Bright's disease in man.[1, 2] Previously we demonstrated that severe acute nephritis induced in rats by means of nephrotoxin generally progressed to a chronic disease with ultimate kidney failure.[2, 3] An occasional rat, however, when fed a stock diet, recovered from the acute renal injury, while a number of animals with chronic nephritis of a year's duration still maintained a normal urea clearance value. This variation in the response of individual animals suggested that the course of the disease might be influenced by internal environmental factors, the "milieu intérieur" of Claude Bernard. Since diet has long been considered an important therapeutic measure in the treatment of Bright's disease, and has been shown to affect the clinical picture in one type of experimental renal injury,[4] it seemed desirable to study its influence on the diffuse glomerulonephritis which results from injection of antikidney serum. Accordingly, severe nephrotoxic nephritis was induced in a large group of rats and the course of the renal disease was observed for 10½ months to determine the influence of different diets. Detailed clinical and chemical studies on 48 of the animals in this group are to be reported elsewhere. The present paper deals with the pathological changes found in these rats and in others followed for a shorter period; renal lesions observed in normal rats maintained on one of the experimental diets are also described.

MATERIALS AND METHODS

Nephrotoxic Serum: Antikidney serum was prepared in rabbits by immunization with sterile suspensions of perfused rat kidney. Throughout this study a single serum was used, *viz.* that from

* Received for publication November 19, 1938.

Rabbit 4557 which was always capable of inducing severe nephritis in rats receiving a total of 0.65 cc. per 100 gm. of body weight, in three divided doses given on consecutive days.[5]

Animals: Young hooded rats of the Whelan strain were injected when they weighed about 100 gm. Males and females were distributed equally in the respective diet groups.

Diet: Rats were fed on one of three isocaloric diets. Each ration contained 27 per cent fat, 4 per cent Osborne and Mendel salt mixture No. 1,* and vitamins plus the following constituents: Diet L — 5 per cent protein and 64 per cent carbohydrate; Diet B — 18 per cent protein and 51 per cent carbohydrate; Diet H — 40 per cent protein and 29 per cent carbohydrate. The protein consisted of Lactalbumin, the fat was chiefly Crisco, while the carbohydrate was composed of a mixture of 2 parts Karo powder and 1 part cane sugar. Cod liver oil U.S.P. which made up 5 per cent by weight of each diet contributed part of the dietary fat and vitamins. Brewers yeast 1 gm. (wet) given on alternate days provided further vitamins. Food and water were always available to the rats.

Technical Procedures: The methods used for collecting clinical and chemical data have been previously described.[3] Moribund animals were generally etherized in order to obtain well preserved tissues. Organs were fixed in Zenker's solution and in 10 per cent neutral formalin. Paraffin sections were stained with eosin and methylene blue, and in addition, Mallory's aniline blue stain and McGregor's [6] modification of the Mallory-Heidenhain method were applied to sections of all kidneys. The scharlach R method was used on frozen sections to show fatty changes. Other staining techniques were occasionally employed.

Experimental

Clinical Course of Nephrotoxic Nephritis in Rats Maintained on Different Diets

The course of the experimental nephritis was similar during the first month in all the rats irrespective of the type of diet that was fed.† Severe albuminuria with cylindruria appeared and per-

* Harris product.

† Data presented in abstract, Farr, L. E., and Smadel, J. E., *Proc. Soc. Exper. Biol. & Med.*, 1937, **36**, 472–473.

sisted. Anasarca was present for a variable number of days. Plasma protein values, which were low during the edematous phase, had generally returned to normal 1 month after the injections of nephrotoxin. On the other hand, blood urea nitrogen values and urea clearance determinations remained within normal limits during this early period, except in a few rats which are not included in this report because they succumbed to the acute disease.

The clinical findings in the group of nephritic rats fed Diet L (low protein-high carbohydrate) diverged markedly during the 2nd month from those of the animals fed the other two diets. Seventeen of the 19 animals that received this diet showed either a marked diminution, or, in a few instances, a complete disappearance of urinary abnormalities. Four of these rats were sacrificed at the end of the 3rd month for histological study. Two animals which had excreted urine of a consistently abnormal character died suddenly in the 5th month of the disease without antecedent renal failure. Eight and one-half months after nephritis had been induced, none of the 13 survivors had elevated blood urea or abnormal urea clearance values; moreover, only 1 showed moderate amounts of protein and casts in the urine, 4 had normal urines and the remainder occasionally put forth traces of urinary protein or a few casts. Two of the rats, which had apparently recovered, and 1 of those with slight evidences of nephritis were sacrificed at this time, while 5 of the 10 surviving rats were changed from Diet L to Diet H (high protein-low carbohydrate). During the final 2 months of observation it became apparent that the change to Diet H had had an adverse effect on the diseased kidneys. The single animal in this subgroup with normal urine at 8½ months did not suffer a relapse, but the other rats with low grade or latent nephritis showed an increase in albumin and casts sufficient to warrant a diagnosis of mild or moderate kidney irritation. Exacerbations of this sort did not occur in the rats maintained on the original Diet L.

Every animal fed Diet H (high protein-low carbohydrate) after receiving antikidney serum continued to have marked albuminuria and cylindruria until it died or was sacrificed. Four members of this group were sacrificed 3 months after injection. Only 2 of the 15 remaining rats survived until the experiment was terminated

10½ months after induction of nephritis; moreover, both of these animals were in a terminal phase of chronic progressive nephritis. Among 13 rats dying with renal failure, the average time of survival after injection was 6 months.

The course of the disease in rats fed on Diet B (basal) was similar to that observed in earlier experiments when the animals were maintained on a varied stock laboratory diet.[3] One of the 15 rats in this group recovered clinically during the 2nd month, while 6 showed abnormal urinary contents throughout the experiment but did not develop renal failure. Eight of the 15 rats died of kidney insufficiency; their average time of survival was 5½ months.

Smaller groups of normal rats fed on Diets L and H for several months were sacrificed for histological study. These animals excreted normal urine throughout the period.

Histopathological Observations

The characteristic lesions in rats with acute nephrotoxic nephritis and the chronic nephritis that follows the acute injury have been described.[2] Both glomeruli and tubules are affected in the acute disease. The tubular epithelium shows necrobiotic changes, principally hyaline droplet degeneration, while the outstanding glomerular lesion consists of swelling of the glomerular capillary basement membranes.

Renal Lesions in Rats Fed Diet B

The nephritic animals maintained on this normal basal diet not only followed the same general clinical course as those kept on a stock diet in previous experiments, but also had essentially the same type of kidney damage; hence, only a summary need be given here. Rats that died of progressive nephritis from 68 to 237 days after receiving nephrotoxin had enlarged kidneys with granular surfaces, and many cystic dilated tubules filled with coagulated material were visible macroscopically throughout the cut sections, especially in the corticomedullary region. These changes were most marked in animals with long-standing disease. Microscopic examination showed that practically all the glomeruli were abnormal: those least affected had distorted tufts with irregularly thickened glomerular capillary basement membranes;

in others the epithelium of Bowman's capsule was proliferated and the capsular membrane was thickened, and there were also glomeruli with varying amounts of connective tissue replacement. Glomerular changes of differing degree were always demonstrable in each section, although the number of severely scarred glomeruli was increased in rats with a more chronic disease. Severe damage was found in tubular structures on histological study; extensive dilatation with large hyaline casts, atrophy in areas of interstitial scarring, and epithelial hyperplasia of the remaining functioning units were all observed. The epithelium of the functioning tubules showed various grades of degeneration, including necrosis, in animals killed when moribund. Vascular lesions as well as perivascular and interstitial infiltrations of cells were characteristically present. Rats in this group, which survived the period of the experiment without developing renal failure, even though showing evidence of chronic nephritis until sacrificed, had the same types of kidney lesions, but many functioning renal units remained.

The single animal in this group in which the nephritic process diminished markedly during the 2nd month, and thereafter became negligible, had kidneys with surfaces that were essentially smooth. Moreover, on microscopic examination most of the structural units appeared normal. A rare dilated tubule filled with a hyaline cast, and an occasional small, completely scarred glomerulus could be found. However, fair numbers of glomeruli which appeared capable of functioning, had slight abnormalities in structure. Changes such as irregular thickening of the capillary basement membrane, local dilatation of capillary loops, thickening of capsular basement membrane and, rarely, small crescents were encountered.

The typical macroscopic appearance of a kidney from a rat with progressive nephritis in this diet group is illustrated in Figure 2. Figures 5 and 7 depict characteristic microscopic lesions seen in such kidneys.

Renal Lesions in Rats Fed Diet H

Young normal and nephritic rats thrived when fed the high protein-low carbohydrate diet H; they gained weight faster and grew larger than rats kept on the basal diet B. A normal male

animal weighing 90 to 100 gm. when placed on Diet H usually doubled its weight in 2½ months. The kidneys of such an uninjected control rat weighed 1.3 to 1.4 gm. each and, except for slight hypertrophy, were normal on inspection and on microscopic examination.

In general, similar kidney lesions were found in rats with chronic progressive nephritis maintained on either Diet B or H; certain differences, however, usually made it possible to distinguish between members of the two groups on macroscopic evidence alone. The kidneys of rats in the Diet H group were usually smaller. Thus, the average weight of the left kidneys of 7 rats dying in Group B was 1.6 gm., while the average of 13 rats dying in Group H was 1.2 gm. The cortical surface was more coarsely granular in Group H. Finally, the cut sections of kidneys from rats in Group B contained many more cystic tubules filled with hyaline material than did kidneys of animals in Group H. These differences were more pronounced in rats which succumbed 5 to 7 months after injection but are clearly depicted in the two kidneys obtained at the end of the experiment and illustrated in Figures 2 and 3.

All gradations of glomerular change described in rats of Group B were observed in animals fed the high protein-low carbohydrate diet; however, the number of completely or extensively scarred glomeruli was always greater in the latter group of animals for any given duration of the nephritis.

A more striking difference between the two groups was found in the varying proportions of the several types of tubular lesions. Cystic tubules filled with hyaline material, observed in such abundance throughout the cortex and medulla of rats with progressive nephritis in Group B, were less frequent and smaller in rats of Group H. On the other hand, necrobiotic changes in the tubular epithelium were more striking in sections from Group H; moreover, degeneration was observed in cells of the proximal segment of tubules in animals of Group H which were killed (3 months after nephritis had been induced) before renal failure was imminent. Widespread interstitial fibrosis encompassing destroyed tubules was marked in the kidneys of the rats in Group H and was observed as early as 3 months after injection of antikidney serum. In addition, hypertrophy and hyperplasia of the epithelium

of remaining functioning tubules were also significantly greater in rats of Group H. Urochrome pigment was conspicuous in the tubular epithelium of rats with terminal anemia irrespective of the diet group. Vascular lesions and perivascular cellular infiltrations occurred with equal frequency and intensity in kidneys of rats that died in the two groups.

In general, glomeruli and tubules which had been damaged by nephrotoxin apparently were unable to undergo repair when the rats were fed the high protein-low carbohydrate diet. Instead, progressive destruction of kidney substance, with connective tissue replacement, proceeded at a more relentless pace than in rats fed the basal diet. Macroscopic and microscopic changes characteristically found in nephritic rats which died while being fed the H Diet are represented in Figures 3, 6 and 8.

Renal Lesions in Rats Fed Diet L

Diet L was sufficiently well balanced to sustain life and to permit a retarded growth of rats, but various abnormalities were observed in animals maintained on it. For example, both untreated and nephritic rats fed Diet L failed to attain normal weight or stature; their fur continued to be soft and short even after the adult state had been reached; and finally, renal and hepatic lesions were consistently observed. It is necessary to present a description of the characteristic pathological changes found in young normal rats maintained on this diet for several months before attempting to evaluate the lesions attributable to nephrotoxin.

A young female rat (PN–13), weight 66 gm., was fed a stock diet for 2 weeks while repeated urine specimens were obtained; no urinary albumin, blood or casts were demonstrable. The animal was then transferred to Diet L. During the next 2½ months this animal gained from 100 to 130 gm. and continued to excrete normal urine.

The kidney, examined at the end of the period, weighed 0.6 gm. and had scattered shallow depressed scars on the cortical surface. On cut section, the corticomedullary region showed numerous, slightly raised yellow streaks arranged in parallel lines radial to the pelvis (see Fig. 11).

The principal changes found on microscopic examination of this kidney occurred in the areas represented macroscopically by

the yellow streaks. Groups of moderately dilated tubules were lined by low cuboidal epithelium, often so thinned as to resemble the connective tissue cells which lay outside the thickened and hyalinized tubular basement membrane. The lumens of dilated tubules generally contained refractile material, sometimes arranged in concentric rings, which stained very faintly with the acid dyes and failed to stain differentially with scharlach R or iodine. Remnants of a brush border could still be identified in certain of the affected proximal convoluted tubules. In the same area, atrophic tubules lined by epithelium with basophilic cytoplasm and with collapsed lumens were present. Their basement membranes were also hyalinized and surrounded by scar tissue. Less drastic changes were demonstrable in other proximal tubules of the corticomedullary area. In some, only a single epithelial cell was degenerated or necrotic, while in others many were affected and cast off cells lay loose in the lumen. Fat was often demonstrated in such damaged cells by scharlach R stains. Regeneration of epithelium was observed and occasionally resulted in an irregular stratification of cells protruding into the lumen. Thickening of the tubular basement membrane or increase in interstitial connective tissue was not conspicuous here. Figure 12 illustrates the microscopic findings in the corticomedullary region of this kidney.

Most of the glomeruli appeared normal but a few had changes in the tuft and occasionally extensive scarring was observed. Damaged glomeruli were usually found near diseased tubules and occasionally could be shown to connect with one of them. Vascular lesions were not found, and interstitial collections of lymphocytes, when present, were small and limited to the corticomedullary scars.

An extreme fatty change was present throughout the liver. The least affected parenchymatous cells, about the portal spaces, contained fat globules, while in the central portion of the lobules the cytoplasm of individual cells stained a homogeneous red with scharlach R.

The renal and hepatic injury which occurred in all young rats fed Diet L for several months varied in degree. Rat PN–13, described above, was one of the most severely affected of the group. On the other hand, Rat PN–7, which seemed to recover

from nephrotoxic nephritis, had a minimal amount of the kidney damage attributable to the low protein-high carbohydrate diet when sacrificed 3 months after injection of antikidney serum.

A young male rat (PN–7) weighed 55 gm. when nephrotoxin was administered and Diet L was started. The acute nephritis began to subside in several weeks and the urine was normal 4 weeks after nephritis had been induced. The animal weighed 94 gm. when sacrificed 3 months after injection. The kidneys, which weighed 0.5 gm. each, were apparently normal on macroscopic examination but showed glomerular lesions throughout the microscopic section. Practically all of the tuft capillaries contained blood but their walls were significantly thickened by an increased width of the basement membranes. About 20 per cent of the tufts were lobulated or otherwise distorted, and approximately 10 per cent of them were adherent to the capsular epithelium in one or more places; nevertheless, well developed crescents were not seen. A few tufts contained scattered cells, apparently epithelial in origin, with large, bright eosin-staining granules in their cytoplasm. Scattered small areas in the corticomedullary region contained dilated tubules, some of which were filled with laminated pale staining material similar to that seen in Rat PN–13; a few atrophic tubules were also present in these areas. An occasional atrophic tubular structure in the cortex was seemingly related to the pathological areas at the junction of cortex and medulla. In addition, tubules containing hyaline casts were occasionally encountered. Vascular lesions and interstitial cellular infiltrations were not observed.

The liver was the seat of mild fatty change. Microscopic sections of the heart were normal.

The kidneys of rats fed Diet L for 8 to 10½ months after nephritis had been induced with nephrotoxin were similar to those of members of the group sacrificed at 3 months. The lesions attributed to the diet were, however, less obvious at 10½ months. The kidneys of only a few of the animals had macroscopic yellow streaks in the corticomedullary region, which on microscopic examination appeared to be dilated tubules filled with poorly staining material. Narrow bands of old connective tissue arranged radially were present in this portion of all the kidneys; these were often conspicuous but sometimes appeared only once or twice in the

entire section. Five to 10 per cent of the glomeruli in the kidneys from older animals were represented by small contracted scars; in addition a few had moderate hyperplasia of the epithelium of Bowman's capsule. The majority of glomeruli appeared to be functioning but showed abnormalities of the tufts such as described in Rat PN–7. The capillaries of the tufts were often dilated in places and contained puddles of blood; these frequently occurred at the border of the tuft. Scattered atrophic tubules were present throughout the cortex. Vascular lesions and interstitial cellular infiltrations were not present in the kidneys of the group of older rats, nor were generalized vascular changes observed.

The livers of the animals sacrificed at the later date showed about as much fatty change as did those examined earlier; certain of the rats also had cirrhosis.

There was no consistent histological difference detected between the kidneys of rats maintained on Diet L throughout the experiments and those of rats transferred from Diet L to Diet H 8½ months after injection of antikidney serum. Fatty change in the livers of rats in this last group, however, was slight when present.

Two rats, in Diet Group L, failed to make a clinical recovery during the 2nd month and died 134 and 139 days, respectively, after nephritis had been induced. Neither of these animals had a significant reduction of kidney function at any time, but their urine contained 1 to 3 gm. of protein per 100 cc. and numerous casts until the end. Kidney lesions in these 2 rats were more extensive than in other members of the group but hardly severe enough to account for the early death. Changes in the tufts, crescent formation, complete scarring of glomeruli, numerous dilated tubules filled with hyaline casts and degenerative changes in tubular epithelium suggested the picture seen in nephritic rats fed Diet B. Both livers were heavily laden with fat. The death of these 2 animals probably depended on the combined effect of severe chronic nephritis and poor diet.

These histopathological observations agree with clinical data and indicate that rats with severe acute nephrotoxic nephritis tend to recover when maintained on a low protein-high carbohydrate diet. The tubular changes characteristic of the acute nephritis appear to be reversible for the most part when circumstances are propitious. Glomerular lesions, on the contrary, do not disappear;

but notwithstanding the presence of residual changes, most of the glomeruli continue to function.

Hematuria in Rats with Chronic and Latent Nephritis

Pearce [7] held that hematuria was not a result of injury with nephrotoxin but depended on other factors in antikidney serum. We have also emphasized this point in reference to the acute syndrome induced by nephrotoxin.[2] Present observations offer no reason for changing our views on hematuria in the acute nephritis but do indicate that renal bleeding may occur in rats after the acute phase of renal injury has passed.

Hematuria, observed in 7 rats, was generally of the intermittent type and when it occurred was obvious macroscopically. Two rats in Group L showed red cells in the urine for the first time after they had been fed the low protein-high carbohydrate diet for 9 and 10 months, respectively; moreover, there had been neither casts nor more than a trace of albumin for months before the appearance of blood in the urine. Onset of hematuria in the 4 rats of Group B occurred in the 3rd (2 animals), 4th and 7th months and, in a single animal of Group H, hemorrhage was detected during the 7th month on one occasion.

Hematuria in these animals seemed to have no adverse prognostic significance for all of the rats lived throughout the experiment. No evidence of an infectious process which might have accounted for the bleeding was found in any of the kidneys, and cultures of renal tissue made at the time of autopsy were bacteriologically sterile. Dilated capillaries near the margins of diseased glomerular tufts were observed in rats of all three diet groups, and rupture of such diseased capillaries seems a likely explanation for the intermittent hematuria.

Generalized Vascular Lesions in Rats with Chronic Progressive Nephritis

Thickening and hyalinization of the walls of small arteries and of arterioles occurring in various organs of rats which succumbed to chronic progressive nephritis were described and illustrated in an earlier report.[2] Additional vascular changes, such as the presence of fat or of calcium in the media and a reduplication of the internal elastic membrane of coronary arteries, and perivascular

TABLE I

Extrarenal Vascular Lesions in Rats that Succumbed to Nephritis

Diet (protein)	Number of rats	Average time of survival	Heart			Pancreas			Brain			Intestine			Liver			Testicle		
			A	C	T	A	C	T	A	C	T	A	C	T	A	C	T	A	C	T
L (low)	2*	4½ mo.			0/2			0/0			0/2			0/0			0/2			0/0
B (basal)	7	5½ mo.	2/7	6/7	7/7	1/4	4/4	4/4	0/5	4/5	4/5	1/1	0/1	1/1			0/7			0/2
H (high)	13	6 mo.	3/13	7/13	9/13	0/9	2/9	2/9	0/12	2/12	2/12	1/1	0/1	1/1	0/11	6/11	6/11	0/7	3/7	3/7

A = Acute lesions
C = Chronic lesions
T = Total number of rats in each group showing lesions of acute or chronic variety or both
The numerator of each fraction indicates the number of rats with lesions; the denominator the number of organs examined.
* Rats did not have renal failure immediately before death but had severe chronic nephritis. Death probably resulted from a combination of nephritis and poor diet.

cellular collections in the pancreas were recorded. The occurrence of chronic generalized vascular lesions has also been noted in the present group of rats which died after long-standing nephritis. Their distribution is enumerated in Table I.

Fibrous myocarditis and encephalomalacia which apparently depended on these chronic vascular lesions were found in 13 of 22 rats with chronic nephritis terminating in death; myocardial scarring occurred 5 times in Group B and 3 times in Group H, while encephalomalacia was noted in 3 members of the former group and in 2 of the latter.

In addition to the chronic extrarenal vascular lesions, other changes of a more acute nature were observed, especially in the heart and intestine. These consisted of proliferation and swelling of endothelium, fibrinoid degeneration in the muscle coat, and pyknosis of muscle nuclei. Table I records the frequency of acute vascular lesions such as are illustrated in Figures 13 and 17.

Vessel changes in the heart, brain and pancreas, as well as myocarditis, were found in 1 of the animals of Group B which survived to the end of the experiment; however, its renal function had been depressed for a month before it was sacrificed. Renal insufficiency was present in both rats of Group H when they were sacrificed 10½ months after injection of nephrotoxin. One had vascular lesions in the heart, brain and testicle and also myocarditis, the other had only coronary abnormalities. None of the rats in Group L which were sacrificed during the experiment, or at its end, had generalized vascular lesions.

Acute Myocarditis and Enteritis in Rats with Renal Failure

An acute focal necrosis of cardiac muscle fibers was frequently seen in rats dying with chronic renal insufficiency. These lesions could usually be identified macroscopically as yellow flecks beneath the epicardium and endocardium. On microscopic examination some groups of degenerated cardiac muscle cells were represented by necrotic débris while others still retained their general outline. The cellular reaction in and about such areas was in the majority of cases mononuclear in type but occasionally consisted of polymorphonuclears or, rarely, was entirely absent. In certain areas cellular collections surrounded muscle fibers which retained their striations. Large mononuclear cells were the prin-

cipal elements in these collections, but an occasional multinucleated cell was seen. Acute lesions, when present, were usually numerous; one section contained nine foci. The myocardium pictured in Figure 16 contained many affected areas. Acute myocarditis occurred in 5 of the 7 rats in Group B and in 9 of the 13 animals in Group H that died with progressive nephritis. Three of the former animals and 2 of the latter had, in addition, old scarring of the myocardium.

Fibrinoid swelling of the ground substance was found in 4 hearts which contained other acute lesions; 2 rats each were involved in Groups B and H. An area of intense fibrinoid degeneration is shown in the left lower portion of Figure 16, while a milder type of reaction is discernible in the area of focal myocarditis illustrated in Figure 15.

Acute myocardial lesions occurred in 14 of the 20 rats with progressive nephritis and protracted renal insufficiency terminating in death. On the contrary, this type of cardiac involvement was not observed in rats of Groups B and H that survived throughout the experiment even though some of these animals showed lowered urea clearance values during the later months of observation; moreover no such lesions were detected in any rat of Group L. McJunkin *et al.*[8] described similar foci of acute myocardial necrosis in nephrectomized rats which were injected with large amounts of phosphate. Those authors do not, however, emphasize the occurrence of swelling and proliferation of endothelium in small coronary arteries and capillaries and these changes, which were usually prominent in our rats with acute myocarditis, may have played some part in the formation of foci of necrosis.

Typhlitis was a contributing cause of death in 2 animals with renal failure; 1 in Group B died on the 198th day and the other in Group H survived 136 days after nephritis was induced. In both instances the cecum showed macroscopic subserosal ecchymoses, edema and hemorrhage of the wall, pseudomembranous exudate covering the mucosa, and blood in the lumen. Figure 14 illustrates such a lesion. Vascular changes of the acute variety were conspicuous near the border of the involved areas (see Fig. 13) and probably were responsible for the intestinal lesion. Goldblatt[9] has recently reported that intestinal bleeding is one

of the cardinal signs of the malignant phase of hypertension in dogs. While blood pressure readings were not made in the present experiment, hypertension was previously demonstrated in rats with chronic progressive nephritis of nephrotoxic origin.[3]

Discussion

The experiments here presented demonstrate that the course of the nephritis in rats which follows injection of antikidney serum is significantly influenced by at least one internal environmental factor, namely, diet. Clinical evidence of renal disease rapidly subsided in almost all young rats placed on a low protein-high carbohydrate diet after severe nephrotoxic nephritis had been induced. Moreover, the acute tubular injury resolved without permanent damage and only moderate residual changes were present in the glomeruli. Renal failure generally occurred within a year, when nephritic rats were maintained on a high protein-low carbohydrate diet, and progressive destruction and scarring of both tubules and glomeruli was observed histologically. More than half the nephritic rats fed a basal diet developed kidney failure and these also had histological lesions indicative of progressive disease. The clinical and pathological features of the nephrotoxic disease were the same in rats nourished with this last diet as in rats fed a mixed stock diet.

While the glomerular lesions found in the fatal cases of nephritis in animals fed either the high protein or basal diets were essentially alike, the tubular changes differed in certain important respects. The marked dilatation of proximal tubular segments in kidneys of Group B rats suggests that the nephron had been rendered functionless through occlusion of the loop or of the distal tubule by scarring or plugging. Degenerative changes were found in the proximal tubular epithelium of kidneys in Group H even when the animals were sacrificed relatively early in the disease; and, in addition, obliterative scarring of proximal tubules was characteristically present in kidneys of the rats that died. It thus appears that one of the most significant effects of diet on the course of nephrotoxic nephritis involves the response of the acutely damaged tubular epithelium. There was a tendency towards recovery from tubular injury when a low protein-high carbohydrate diet was fed, while progressive involvement of the distal tubular

segment was characteristically found when a basal diet was given, and, finally, destruction of the proximal tubule was outstanding in nephritic rats maintained on a high protein-low carbohydrate diet. The studies of Richards and Walker [10] on selective action of different portions of the kidney tubule in frogs and necturi suggest an approach to an explanation of our observations in rats, but the exact factors responsible for the recovery of the nephron or destruction of its proximal or distal segment are not clear. The response of the glomerular capsular epithelium was similar to that observed in the remainder of the nephron. Obliteration of the capsular space by crescent formation occurred relatively infrequently in rats of Group L notwithstanding the fact that residual changes in the glomerular capillary bed were common. In contrast to this, marked proliferation of the epithelium of Bowman's capsule with connective tissue ingrowth into the glomerular tuft was conspicuous in the kidneys of rats in both Groups B and H.

Medlar and Blatherwick [11] have commented on the similarity of the renal picture in chronic dietary nephritis observed in rats with unilateral nephrectomy which were maintained on rations rich in animal protein, and the terminal phase of nephrotoxic nephritis in our rats fed a mixed stock diet. The increased susceptibility of male rats to dietary nephritis noted by these workers was less clear-cut in the present experiments; nevertheless 5 of the 7 rats in Group B and both of the animals in Group H that survived the experiment were females.

Generalized vascular changes of both acute and chronic varieties and the visceral lesions that stem from them were encountered only in rats which developed chronic progressive nephritis and renal insufficiency. Diet apparently influenced production of the extrarenal disease only indirectly by way of its effect on the injured kidney. Differences in the frequency of vascular changes were observed in Diet groups B and H, but probably were not significant. Animals that lived throughout the experiment were approximately 380 days old when sacrificed. The failure to find chronic vascular disease or fibrous myocarditis in our surviving animals with normal urea clearance values agrees with the experience of Wilens and Sproul.[12, 13] These authors observed cardiovascular lesions in more than half the members of a large group of senile rats. However they state regarding the heart

that "almost all of the changes described make their appearance late in the second year of life and do not attain their maximum incidence until well into the third year." Furthermore, they observed few vascular lesions before the 700th day of life. It may well be that the process of aging and the concomitant development of chronic cardiovascular disease are merely accelerated in rats with severe nephritis.

It should be emphasized that rats used in these studies were all of a black and white hooded strain designated as Whelan stock. Experiments to be reported by Swift and Smadel have indicated that rats of the Wistar and Evans strains respond somewhat differently to the effect of nephrotoxin and diet.

Conclusion

In rats of the so-called Whelan strain the chronic nephritis which follows the administration of antikidney serum can be markedly influenced by isocaloric diets containing different proportions of protein and carbohydrate.

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DESCRIPTION OF PLATES

PLATE 38

FIG. 1. Cortical and cut surfaces of kidney from Rat 4 L. Rapid recovery from acute nephrotoxic nephritis occurred on the low protein diet; albuminuria had diminished to a faint trace and casts were absent a month after injection of antikidney serum. Autopsied 10½ months after onset of acute nephritis. Left kidney, weight 1 gm. × 3.

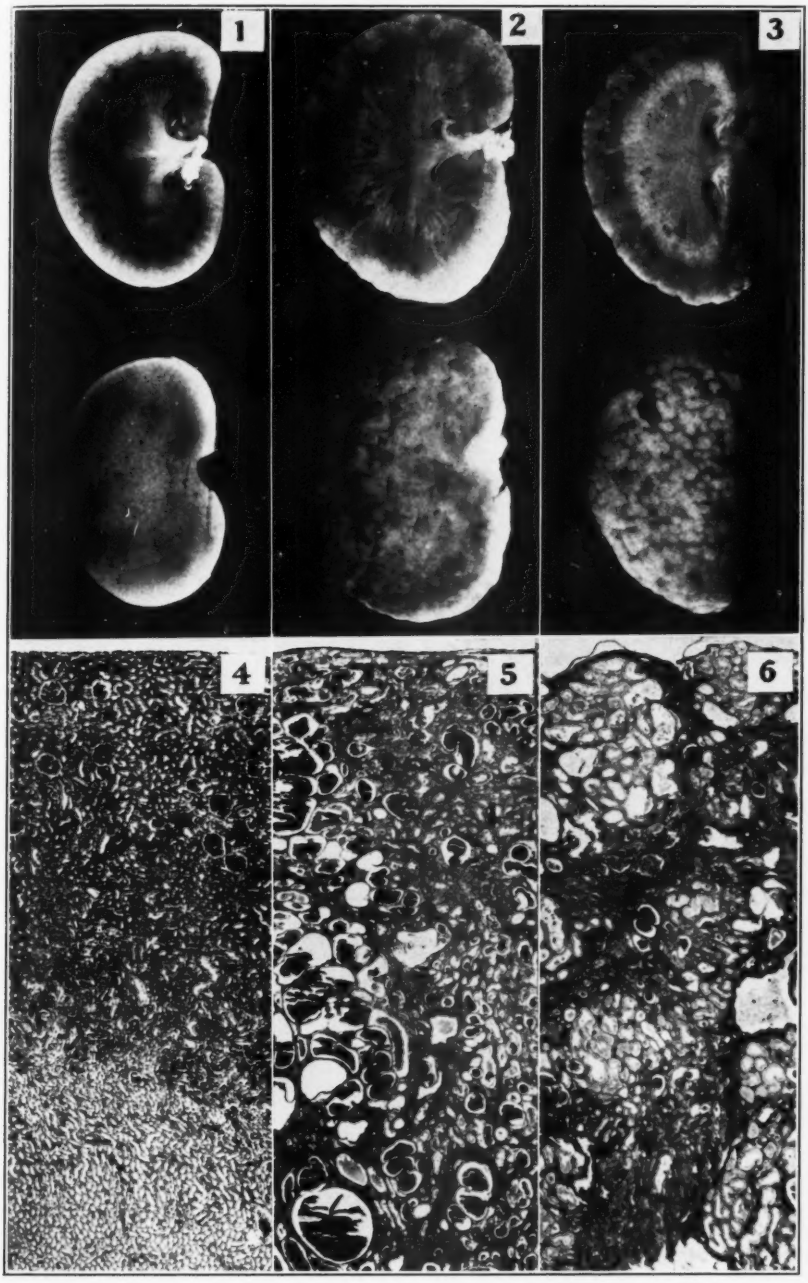
FIG. 2. Kidney of Rat 8 B in Diet group B. Severe albuminuria and cylindruria continued from the time of induction of acute nephritis until the rat was sacrificed 10½ months later; final urea clearance value was within the lower limits of normal. Kidney weight 2.1 gm. × 3.

FIG. 3. Kidney of Rat 3 H fed the high protein diet for 10½ months after injection of nephrotoxic serum. Severe nephritis was evident throughout and a depressed urea clearance was observed during the 2 months preceding sacrifice. Kidney weight 1.7 gm. × 3.

FIG. 4. Rat 4 L. Few abnormalities are detectable in the section of kidney at this magnification. Mallory's aniline blue stain. × 35.

FIG. 5. Rat 11 B, basal diet group, died with renal failure 7 months after onset of nephritis. Extensive destruction of tubules and distortion of glomeruli are evident, but the most conspicuous abnormality is the markedly dilated tubules filled with hyaline material. Mallory's aniline blue stain. × 35.

FIG. 6. Rat 5 H, high protein diet, succumbed to kidney insufficiency 5½ months after receiving nephrotoxin. Glomerular damage is similar to that found in Fig. 5. Nests of hypertrophic tubules constitute the bulk of the substance. Extensive areas of interstitial scarring have replaced destroyed tubules. Mallory's aniline blue stain. × 35.



Smadel and Farr Effect of Diet on Rats with Nephritis

Plate 39

Figs. 7 and 8. Higher magnification of the same sections illustrated in Figs. 5 and 6 respectively. × 150.

Fig. 9. A higher magnification of the section shown in Fig. 4. The tubules are not significantly altered and the glomeruli appear capable of functioning. Moderate irregular thickening of the glomerular capillary basement membranes is apparent. Some distortion of the glomerular tuft on the right is to be seen, while slight proliferation of the capsular epithelium and a small capsular adhesion can be observed in the glomerulus on the left. × 175.

Fig. 10. An area of encephalomalacia in the cortex of the parietal lobe of Rat 13 B, basal diet group, which died of renal failure 5½ months after nephritis had been induced. Eosin-methylene blue stain. × 70.

Smadel and Farr

Effect of Diet on Rats with Nephritis

Plate 40

Fig. 11. Kidney of Rat PN 13, an uninoculated control animal which was fed the low protein diet for 3 months. The pale streaks in the corticomedullary region were yellow in the fresh specimen. × 4.

Fig. 12. Higher magnification of Fig. 11, showing degenerated tubules with casts in the corticomedullary region. Eosin-methylene blue stain. × 250.

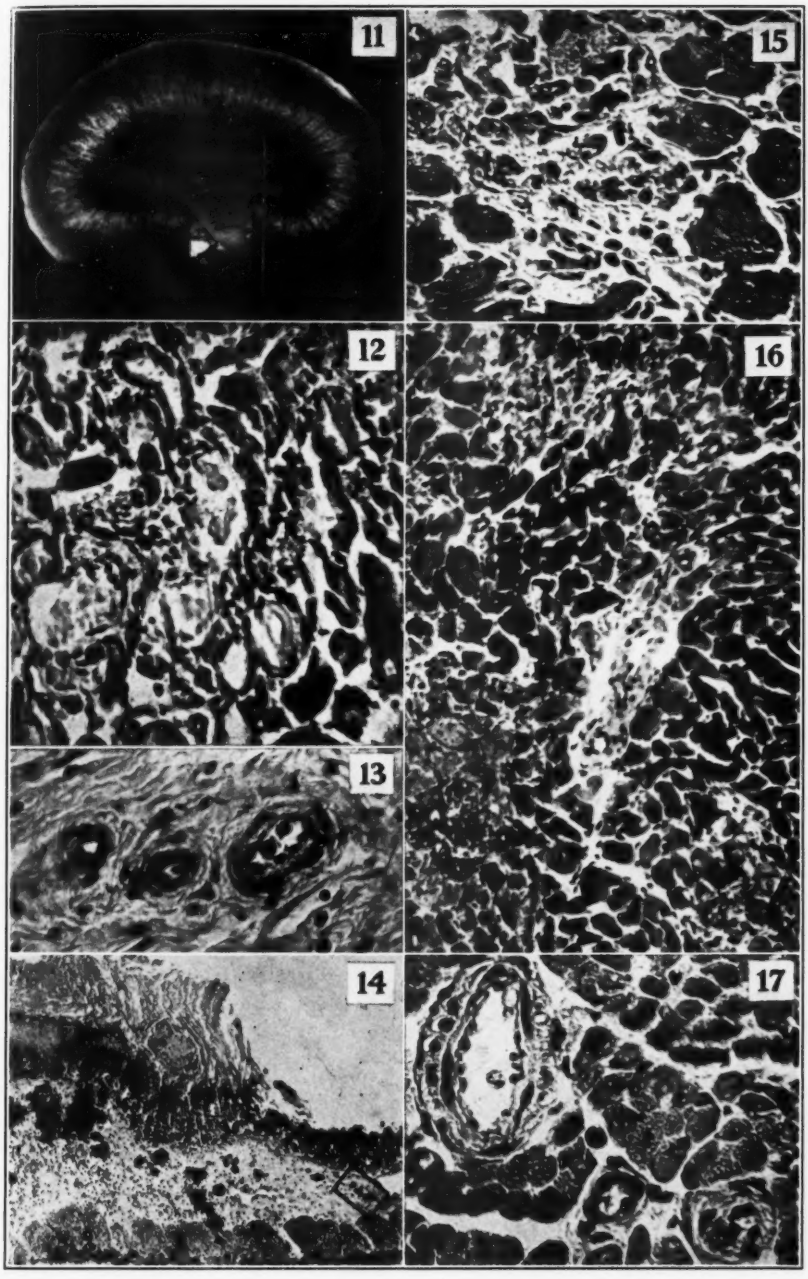
Fig. 13. Section of the cecum of Rat 8 H, which was fed the high protein diet and which died 7 months after the injection of nephrotoxin. An acute necrotizing process is present in all three vessels. These vessels can also be seen at the extreme right in Fig. 14. Eosin-methylene blue stain. × 210.

Fig. 14. Same as Fig. 13. Hemorrhage and edema in the submucosa with a fibrinous exudate covering the necrotic mucosa are shown. × 30.

Fig. 15. Section of the heart of Rat 15 B, basal diet group, which succumbed with nephritis in the 7th month. A small area of focal necrosis of muscle fibers and infiltration with mononuclear cells is seen. Eosin-methylene blue stain. × 450.

Fig. 16. Same as Fig. 15. Multiple focal lesions. An area of fibrinoid change surrounded by muscle fibers showing fresh necrosis is present at the lower left. Areas of necrosis with cellular infiltrations are shown at the lower right and upper portions of the microphotograph. Thickening of vessel walls was also present in other portions of the section. Eosin-methylene blue stain. × 175.

Fig. 17. Heart of Rat 10 B, basal diet group, which died with nephritis after 68 days. Swelling and vacuolation of cells of the media are prominent in the large vessel on the left. Hyperchromatic enlarged cells are seen in the wall of the small vessel in the lower center as well as swelling of the intimal cells. The vessel at the lower right is completely occluded by intimal proliferation. Eosin-methylene blue stain. × 450.



Smadel and Farr Effect of Diet on Rats with Nephritis

CARCINOMA OF THE PANCREAS *

An Analysis of Forty Autopsies

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In a previous communication [1] we analyzed 40 cases of primary carcinoma of the biliary system encountered among 6050 autopsies on persons over 1 year of age performed by the staff of the Department of Pathology of the Charity Hospital of Louisiana, New Orleans, between Jan. 1, 1931, and Oct. 6, 1937. The present study is based on 40 cases of primary carcinoma of the pancreas encountered in the same series of autopsies. This analysis, similar to the previous one, is principally concerned with the site of the primary growth, its structure, and its spread locally and to distant parts. The clinical manifestations and their duration, and the immediate cause of death in the afflicted patients are also touched upon briefly.

Race, Sex and Age: Twenty-two of the patients were negro (18 male, 4 female) and 18 were white (17 male, 1 female). One of the patients died in the 3rd, 1 in the 4th, 7 in the 5th, 8 in the 6th, 16 in the 7th, and 7 in the 8th decade of life. The youngest patient was 26 and the oldest 80 years of age (Tables I and II).

Site and Structure of Neoplasms: Of the 40 neoplasms, 31 were situated in the head and 9 in the tail of the pancreas. Of the neoplasms primary in the head, 21 were 8 to 10 cm. in diameter or larger. The diameter of the remaining 10 varied from 2 to 6 cm. Of those primary in the tail, 4 were 2 to 5 cm. in diameter, and the remaining 5 were larger.

Whether the growths were primary in the head or in the tail of the pancreas, the gross appearance was practically the same. An infiltrating, firm, gray or yellow-white mass sprinkled with opaque yellow and red or brown areas usually formed the bulk of the growth. At the periphery the mass blended with the pancreatic tissue along a zig-zag line. Sectioning the growth fre-

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TABLE I

Data on Patients with Carcinoma Primary in the Head of the Pancreas

Number of case	Age	Sex and race	Spread		Clinical manifestations	Operation	Duration of illness	Cause of death
			Regional	Distant				
	yrs.						*mos.*	
H–1 '34–717	26	M C	Lymph nodes	Lymph nodes	Loss of weight, jaundice	None	7	Carcinoma
H–2 '32–312	33	M W	Lymph nodes, peritoneum	Pleurae, heart	Loss of weight, jaundice	Cholecystectomy	4	Carcinoma
H–3 '34–1248	41	F C	None	None	Jaundice	Choledochoduodenostomy	6	Carcinoma
H–4 '36–710	45	M C	Lymph nodes, duodenum	Liver	Loss of weight, jaundice	Laparotomy	4	Carcinoma
H–5 '32–873	47	F C	None	None	Loss of weight, jaundice	Laparotomy	6	Carcinoma
H–6 '33–316	47	F W	Lymph nodes	Liver, lungs	Loss of weight, jaundice	Laparotomy	3	Carcinoma
H–7 '35–690	47	M C	None	None	Loss of weight	Laparotomy	2	Carcinoma
H–8 '35–1110	47	M C	Lymph nodes, peritoneum	Liver, lungs	Loss of weight, jaundice, ascites	None	8	Carcinoma
H–9 '33–1151	49	M C	Lymph nodes, duodenum	Liver	Loss of weight, jaundice	Laparotomy	3	Carcinoma
H–10 '33–845	50	F C	Lymph nodes, duodenum	None	Loss of weight, jaundice	Laparotomy	5	Carcinoma
H–11 '34–940	50	M W	Lymph nodes, duodenum	Liver	Ascites, jaundice, cholelithiasis	None	4	Peritonitis
H–12 '32–248	51	M W	Lymph nodes	Liver	Ascites, jaundice, cholelithiasis	None	24	Carcinoma
H–13 '36–1495	53	M C	Lymph nodes	Liver	Loss of weight	None	4	Diffuse pneumonia
H–14 '36–1028	55	F C	Lymph nodes, peritoneum	Liver	Jaundice, hemoptysis	Laparotomy	14	Pulmonary tuberculosis, hemorrhage
H–15 '34–1302	55	M W	Lymph nodes	None	Jaundice	Cholecystgastrostomy	3	Gastric hemorrhage
H–16 '33–679	61	M W	Lymph nodes, duodenum	Liver	Jaundice, cholecystitis	Cholecystostomy	2	Carcinoma
H–17 '33–566	61	M W	Lymph nodes, duodenum	Liver	Loss of weight, jaundice	Cholecystoduodenostomy, gastroenterostomy	4	Gastric hemorrhage

H–18 '35–739	62	M W	Lymph nodes	Liver	Jaundice	Cholecystenterostomy	7	Carcinoma
H–19 '36–1440	62	M W	Lymph nodes, peritoneum	None	Loss of weight, ascites	None	8	Focal pneumonia
H–20 '32–720	63	M W	Lymph nodes, peritoneum	None	Loss of weight	Cystotomy	8	Prostatic enlargement, pyelonephritis
H–21 '37–498	65	M C	Lymph nodes, peritoneum	Liver	Ascites	None	3	Typhoid fever
H–22 '34–590	66	M C	Lymph nodes	Liver	Loss of weight, jaundice, ascites	None	2	Carcinoma
H–23 '36–465	66	M W	Lymph nodes, peritoneum	None	Loss of weight, jaundice, ascites	None	7	Carcinoma
H–24 '33–1012	66	M W	Lymph nodes	Liver	Loss of weight, ascites	Prostatectomy	6	Tertian malaria
H–25 '32–568	67	M C	Lymph nodes, peritoneum	None	Loss of weight, jaundice	Gastroenterostomy	8	Carcinoma
H–26 '33–574	69	M C	Lymph nodes, duodenum	Liver	Loss of weight, jaundice	None	2	Carcinoma
H–27 '31–292	70	M W	Lymph nodes, peritoneum	Liver, lungs, kidney (left)	Ascites	None	3	Carcinoma, aortic aneurism
H–28 '32–52	70	M C	Lymph nodes, duodenum	Liver	Jaundice	None	2	Carcinoma
H–29 '36–564	70	M C	Lymph nodes	Liver	Loss of weight	None	1	Carcinoma
H–30 '33–775	72	M W	None	None	Jaundice	None	7	Carcinoma
H–31 '35–456	80	M C	Lymph nodes, peritoneum	Liver, pleurae, pericardium	Loss of weight, ascites	None	5	Carcinoma

TABLE II

Data on Patients with Carcinoma Primary in the Tail of the Pancreas

Number of case	Age	Sex and race	Spread		Clinical manifestations	Operation	Duration of illness	Cause of death
			Regional	Distant				
	yrs.						*mos.*	
T–1 '36–1075	50	M W	Lymph nodes, peritoneum, spleen	Liver, lungs, heart, kidneys, adrenals	Loss of weight	None	5	Carcinoma
T–2 '36–496	51	M C	Lymph nodes, peritoneum	Liver	Loss of weight	Duodeno-jejunostomy	6	Carcinoma
T–3 '32–913	60	M W	Lymph nodes, peritoneum	Liver	Loss of weight, jaundice, ascites	None	8	Carcinoma
T–4 '34–161	62	M C	Lymph nodes, peritoneum, spleen	Liver, adrenals	Loss of weight	Laparotomy	3	Focal pneumonia
T–5 '32–695	65	M C	Lymph nodes, peritoneum	None	Loss of weight	None	6	Carcinoma
T–6 '37–319	66	M W	Lymph nodes, peritoneum	Liver	Jaundice, ascites	None	4	Carcinoma
T–7 '34–1049	67	M C	Lymph nodes, peritoneum	Liver	Loss of weight, ascites	None	7	Carcinoma
T–8 '35–810	75	M C	Lymph nodes, peritoneum	Liver, lungs, pericardium, kidneys	Loss of weight, ascites	None	5	Carcinoma
T–9 '32–44	76	M W	Lymph nodes	Liver, lungs	Loss of weight	None	1	Thrombosis, vena portae

quently elicited a gritty sensation. The cut surfaces disclosed a pattern similar to the external appearance with occasional small cavities containing fluid and necrotic or hemorrhagic débris.

All the growths, whether primary in the head or in the tail, were columnar cell carcinomas. There was, however, some variation in the cellular morphology of the parenchyma and in the amount of stroma. Usually low cuboidal or tall columnar neoplastic cells formed acinar or tubular structures and were embedded in a scant loose or dense fibrous connective tissue stroma. In some instances the neoplastic tissue was distributed amid fairly intact pancreatic tissue; in others it was within masses of connective tissue rich in collagenous bundles containing remains of scattered small acini and dilated ducts. Areas of necrosis and hemorrhage were frequent in the centers of larger tumor masses. In many areas devoid of acini and containing only dilated pancreatic ducts islands of Langerhans were prominent. An occasional island was invaded by neoplastic tissue (Fig. 1). In some of the neoplasms the cells were tall columnar with pale staining cytoplasm and basally located nuclei. In others the cells were low columnar or cuboidal with a granular or basophilic cytoplasm, and mitoses seemed more frequent. In a few instances the tall columnar cells resembled those lining the large ducts, as if the growth had arisen from them (Fig. 2). In none of the growths did the cellular structure resemble the islands of Langerhans.

Manner of Spread: Local extension of the growth with involvement of regional lymph nodes occurred in 38 of the 40 cases (95 per cent). Carcinoma primary in the head of the pancreas readily invaded the duodenum (8 instances) and that primary in the tail spread over the peritoneum. Metastases were encountered in the liver in 25 instances, in the lungs and pleurae in 6, in the heart and pericardium in 3, in the kidneys in 3, and in the suprarenal glands and the spleen in 2 instances each.

Clinical Course: Obstruction of the common bile duct with varying degrees of jaundice occurred in 22 of the 31 patients with carcinoma in the head of the pancreas, and 10 had ascites. Jaundice occurred in 2 of the 9 patients with carcinoma in the tail of the pancreas, and 4 had ascites. Loss of weight was a prominent feature in practically all instances.

Operations were performed on less than one-half of the patients.

In 38 of the 40 patients the illness lasted from 1 to 8 months, with an average duration of 4½ months. The newgrowth was the principal lesion and the immediate or contributory cause of death in all but 5 patients.

Comment

The older reports on carcinoma of the pancreas are reviewed by Kiefer,[2] who analyzed 33 cases observed at the Peter Bent Brigham Hospital up to the year 1927. Stout[3] reviewed 33 cases recognized at the Presbyterian Hospital in New York City up to 1932. Leven[4] in 1933 analyzed 99 cases at the University of Minnesota. According to Eusterman and Wilbur,[5] approximately 403 cases of pancreatic carcinoma, verified by operation or autopsy, were seen in the Mayo Clinic during the period between 1921 and 1931. The article by Whipple, Parsons and Mullins[6] in 1935 on the surgical treatment of carcinoma of the ampulla of Vater renewed interest in the subject, and since its publication a number of reports have appeared dealing with the clinical aspects of the problem. The most recent are those by Rives, Romano and Sandifer,[7] Brunschwig,[8] Cooper,[9] Whipple,[10] Ransom,[11] and Crile.[12]

In the reports assembled by Leven the ratio of males and females varied from 3:2 to 4:1. In our own series the proportion was 7:1, although in our total series of autopsies the proportion was 2:1. According to Leven carcinoma of the pancreas occurs most frequently in the 5th, 6th and 7th decades of life. This is true for our series, although over half of our patients were in the 7th decade or older.

The primary growth was located in the head of the pancreas about three times more frequently than in the tail. This proportion approximates the observations of others.

No appreciable difference was noted in the gross or microscopic appearance of the growths arising in the head and in the tail of the pancreas. In a number of instances islands of Langerhans were prominent amid the neoplastic tissue, as if spared by the growth. In a few instances, however, the growth invaded some of the islands. The prominence of the islands amid the neoplastic tissue is mentioned by Ewing[13] and also by Warren.[14]

Summary

In 6050 autopsies on persons over 1 year of age there were 40 cases of primary carcinoma of the pancreas.

Males and females were represented in the proportion of 7:1. Twenty-three of the patients were over 60 years of age.

The average duration of illness was 4½ months.

Thirty-one neoplasms were situated in the head and 9 in the tail. All were columnar cell carcinoma.

Carcinoma primary in the head of the pancreas readily invaded the duodenum and that primary in the tail spread over the peritoneum. Metastases were observed in the liver in 25 instances.

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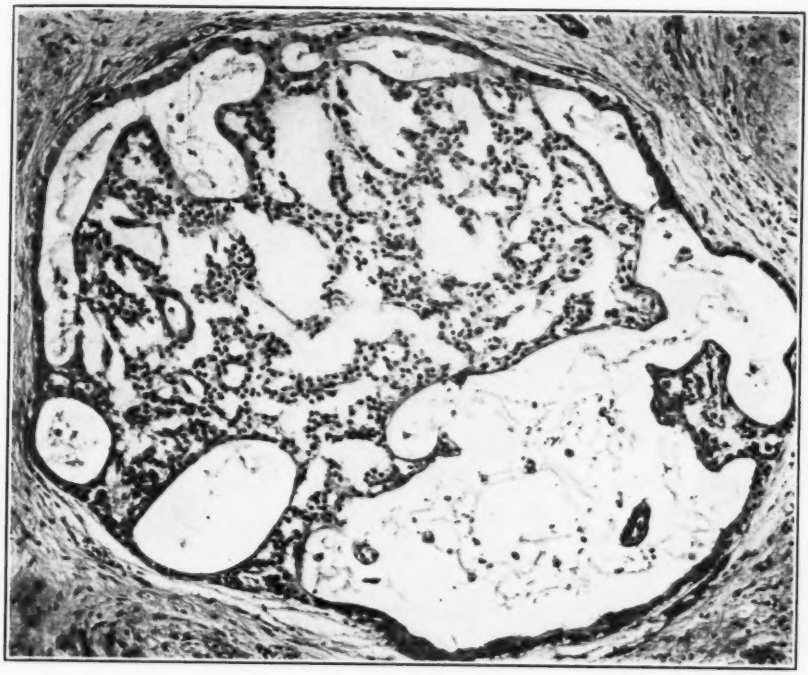
DESCRIPTION OF PLATE

PLATE 41

FIG. 1. H–10. Neoplastic epithelial cells are seen within the island of Langerhans. The cell cords are spread apart.

FIG. 2. T–2. The neoplastic tall columnar cells with their lightly stained cytoplasm and basally placed nuclei resemble the lining cells of the large ducts, as if the growth had arisen from them.



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2

EXPERIMENTS ON THE SOLUBILITY OF HEMOSIDERIN IN ACIDS AND OTHER REAGENTS DURING AND AFTER VARIOUS FIXATIONS *

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Virchow [1] first in 1847 reported on the solubility of blood pigment in acids, finding that the granular intracellular form was soluble in warm concentrated sulphuric acid, but resisted cold sulphuric acid, alkalies and other reagents. The pigment was reprecipitable from its sulphuric acid solution with an excess of ammonia as brown floccules, which after ashing and solution in hydrochloric acid gave the Prussian blue reaction. Previous treatment of the pigment with alkali made it soluble in nitric acid and more quickly soluble in sulphuric.

Following the introduction of the ferrocyanide reaction by Perls [2] in 1867, and of the ammonium sulphide reaction by Quincke [3] in 1880, little attention was paid to the acid solubility of hemosiderin until the publication of Hueck's monograph in 1912. Neumann [4] in 1888 had noted the pigment showed a quite notable resistance to mineral acids. Hueck [5] stated that hemosiderin may require a very thorough treatment with aqueous solutions of acids, less with alcoholic solutions, to dissolve it, and published a table in which hemosiderin was designated as "soluble in acids" without qualification. This table was reprinted and the statements repeated by Hueck in Krehl and Marchand's Handbuch der allgemeinen Pathologie in 1921,[6] and since then the bald statement that hemosiderin is soluble in acids has appeared in various texts on general pathology and on pathological technique (Oberndorfer,[7] Mallory and Wright,[8] Mallory,[9] Schmorl,[10] and von Gierke [11]).

Acid extraction has been used by various workers to remove hemosiderin when studying other pigments. Brown,[12] Seyfarth,[13] and Mayer [14] each used oxalic acid to remove hemosiderin while studying the possible iron content of malaria pigment. Brown and Seyfarth used 12 hours extraction with 2 per cent oxalic acid and

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found iron-reacting pigment afterward. Mayer prolonged this to 18 hours and found the reaction positive in some cases and not in others, but the iron-containing pigment was found only in phagocytes, not in red corpuscles. Glasunow [15] extended the extraction to 24 hours and found no remaining iron. He showed that concentrated oxalic acid did not dissolve malaria pigment in 48 hours and speculated that 12 hours treatment with 2 per cent oxalic acid might be insufficient to remove hemosiderin, but did not try extraction of known hemosiderin. He further noted the

TABLE I

Hemosiderin Remaining in Formalin-Fixed Paraffin Sections after Varying Exposures to Varying Concentrations of Various Acids

Stock acid	Dilution	Time	Temperature (room)	Material	Amount of hemosiderin remaining
	%	*hrs.*	*C.*		
None	—	—	30°	S-6896	++++
Conc. HCl	1	48	30°	S-6896	+++
Conc. HCl	10	48	30°	S-6896	+++
Conc. HCl	20	48	30°	S-6896	o – trace
Conc. H_2SO_4	10	48	30°	S-6896	o
Conc. H_2SO_4	10	24	30°	S-6896	o
Conc. H_2SO_4	10	16	30°	S-6896	Trace
Conc. H_2SO_4	10	8	30°	S-6896	+
Conc. HNO_3	5	48	30°	S-6896	+ – +++
Conc. HNO_3	10	48	30°	S-6896	+ – ++
Glacial acetic	20	48	30°	S-6896	++++
90% Formic	20	48	30°	S-6896	+ – ±

insolubility of hemosiderin in aniline, pyridine and 4 per cent quinine in chloroform and the slow solubility of malaria and formalin pigments in these reagents (3–14 days).

In a study of melanosis of the appendix,[16] I used extraction for 2 hours with 20 per cent sulphuric acid to exclude hemosiderin, and we have generally relied on similar extractions with 20 per cent sulphuric acid or a hydrocholoric acid alcohol containing about 2.5 per cent hydrogen chloride to remove hemosiderin.

In the summer of 1937 a section of bone marrow which had been decalcified 2 or 3 days in a 20 per cent formic acid-sodium citrate fluid was included among several sections being stained for iron by the ferrocyanide method, and a considerable amount of hemosiderin was demonstrated.

This finding raised a question as to the degree and rapidity of solution of hemosiderin in acids. Accordingly, a series of experiments was initiated to explore the problem, primarily in connection with establishing reliable technical procedures for our own use.

In the first experiment a series of sections of a heavily hemo-

TABLE II

Hemosiderin Remaining in Paraffin Sections of Formalin-Fixed Material from a Skin Tumor and a Liver from a Case of Hemochromatosis after Varying Exposures to Various Acids

Stock acid	Dilution	Time	Temperature	Material	Amount of hemosiderin remaining
	%	*hrs.*	*C.*		
Conc. H_2SO_4	10	1	37°	S–6896	+
				A–1228	++
Conc. H_2SO_4	10	2	37°	S–6896	±
				A–1228	+
Conc. H_2SO_4	10	4	37°	S–6896	Trace
				A–1228	Trace
90% Formic	20	8	37°	S–6896	+++
				A–1228	++++
90% Formic	20	16	37°	S–6896	± most brown
				A–1228	+ most brown and in portal area
90% Formic	20	24	37°	A–1228	+ most brown and in portal area
Glacial acetic	20	48	37°	S–6896	++
				A–1228	++ – +++
Conc. HCl	10	8	37°	S–6896	+ part brown
				A–1228	++ part brown
Conc. HCl	..	16	37°	S–6896	± part brown
				A–1228	+ most brown and in portal area
Conc. HCl	..	24	37°	S–6896	± part brown
				A–1228	± most portal

siderotic cutaneous sarcoma, fixed routinely in 10 per cent formalin, was utilized. The paraffin sections were brought to water as usual and then soaked in acid solutions for varying periods. Sections were then washed in water and stained by Dr. Maude Abbott's modification of Perls' reaction (Mallory and Wright,[8] 1924, p. 207), using dilute fuchsin as a counterstain. The results are presented in Table I.

Table I shows that 20 per cent hydrochloric acid for 48 hours at about 30°C., or 10 per cent sulphuric acid for 16 hours, barely removed all the demonstrable iron, and that 10 per cent nitric

TABLE III

Persistence of Hemosiderin in Formalin-Fixed Tissues from Various Cases on Soaking in Sulphuric and Other Acid Solutions

	A–1747 Brown induration of lung (a)					10% Sulphuric acid							
											A–1695		
Time in acid at 25°C.	5% oxalic	20% acetic	HCl alcohol	20% H_2SO_4	10% H_2SO_4	A–1743 Lung CPC(a)	A–1735 Lung CPC(a)	A–1716 Spleen	A–1593 Liver cirrhosis	A–1367 Spleen (b)	Liver Kc(b)	Spleen (c) pulp (b)	Lymph node (c)
Control	+++	+++	+++	+++	+++	+++	++	+++	++	+++	+++	+++	+++
½ hr.	+++	..	+++	+++	+++	+++	++	+++	++	+++	++ (c)	++	+++
1 hr.	++	..	+++	+++	+++	+++	+.++	+++	+	+++	++ (c)	++	+++
2 hrs.	+	..	+++	++ (c)	+++	+ (*)	+	+++	+	+++ (c)	+ (c)	++	+++
4 hrs.	− (c)	..	+++	+ (c)	++	+++	+	++	+	++ (c)	+ (c)	+	+++
8 hrs.	− (c)	..	+++	− (c)	+	+++	−	+.++	±.+	± (c)	± (c)	±.+	++
16 hrs.	− (c)	..	+++	− (c)	±	++	− (c)	+	− (c)	− (c)	± (c)	±	±
1 day	− (c)	+++	+++	−	−	−	−	±.+	−	− (c)	− (c)	−	−
2 days	− (c)	+++	+++										
3 days	− (c)	+++	+++										
4 days	−	+++	++										
5 days		++.+++	++										
7 days		++	+										

Kc = Kupffer cells.
CPC = Chronic passive congestion.
(a) = Some carbon present in addition to hemosiderin.
(b) = + formalin pigment in hemolyzed areas.
(c) = Some brown granular iron-free pigment.
(*) = Unexplained irregularity.

acid and 20 per cent formic acid did not quite remove it all in 48 hours, and that 20 per cent acetic for 48 hours was almost without appreciable effect.

The next experiment was made at 37°C. and showed more rapid removal of demonstrable iron. However, often part of the brown pigment remained, though it failed to give the ferrocyanide reaction. The results are shown in Table II.

Table II shows that 4 hours at 37°C. in 10 per cent sulphuric acid removed practically all of the pigment, that 10 per cent hydrochloric acid or 20 per cent formic acid removed most of the iron from the pigment in 16 to 24 hours at 37°C. but left much brown pigment, and that acetic acid was a little more active in pigment removal or iron removal than at 30°C.

Further human formalin-fixed material from 7 cases was selected for testing to see whether there was any great variation in acid solubility of hemosiderin in various cases. One of these was used also to see whether longer exposure to 20 per cent acetic acid would be effective and to test the solvent action of oxalic acid on hemosiderin.

Table III shows that the limits of persistence of hemosiderin in 10 per cent sulphuric acid at room temperature (25°C.) were 16, 16, 4, 24, 8, 8 and 16 hours for the 7 cases, 4 hours in 20 per cent sulphuric, and 2 hours in 5 per cent oxalic acid in the 1st case. Hydrochloric acid alcohol (2.5 cc. HCl in 100 cc. 70 per cent alcohol) and 20 per cent acetic acid were very slow, still showing some hemosiderin after 15 and 30 days respectively.

Apparently iron-containing pigment in formalin-fixed material is relatively resistant to acids.

The next experiment was to test the solubility of the pigment in various acid-containing fixatives applied to fresh unfixed tissue from the spleens of guinea pigs previously given several intraperitoneal inoculations with sheep erythrocytes. The results are presented in Tables IV, V and VI.

From these tables it seems evident that formalin protects hemosiderin against the solvent action of formic and acetic acids, but not sulphuric, while Zenker's potassium bichromate and corrosive sublimate mixture, which by itself preserves hemosiderin, fails to prevent solution of the iron from the pigment by formic, acetic and nitric acids.

TABLE IV

Amounts of Hemosiderin Shown in One Spleen Fixed by Various Fixing Fluids

Fixing solution	Time	Amount of hemosiderin remaining	Red cells
	hrs.		
10% aqueous formalin, neutral	48	+++	Preserved
10% formalin, 5% acetic in water	48	+++	Laked
10% formalin, 5% formic in water	48	+++	Laked
10 pts. formalin, 90 pts. 95% alcohol	24	+++	Partly laked
10% formalin, 5% acetic in alcohol	24	+++	Laked
10% formalin, 5% formic in alcohol	24	+++	Laked
Helly-Maximow ($HgCl_2$ 6, $K_2Cr_2O_7$ 2.5, H_2O 100, formalin 10)	22	+++	Preserved
Zenker ($HgCl_2$ 6, $K_2Cr_2O_7$ 2.5, H_2O 100, glacial acetic 5)	22	+ brown + green ± brown	Laked
Zenker-formic ($HgCl_2$ 6, $K_2Cr_2O_7$ 2.5, H_2O 100, formic 5)	22	+ green + blue	Mostly laked
Zenker-nitric ($HgCl_2$ 6, $K_2Cr_2O_7$ 2.5, H_2O 100, HNO_3 5)	22	+++ brown	Partly laked

TABLE V

Further Tests of Fixing Solutions on Hemosiderin in One Spleen

Fixing solution	Time	Amount of hemosiderin remaining	Red cells
	hrs.		
Sublimate ($HgCl_2$ sat. aq. 85, H_2O 15)	24	−	−
Sublimate-nitric (sat. aq. $HgCl_2$ 85, H_2O 10, HNO_3 5)	24	−	Laked
Sublimate-acetic (sat. aq. $HgCl_2$ 85, H_2O 10, acetic 5)	24	−	Laked
Sublimate-formol-acetic (sat. aq. $HgCl_2$ 85, formalin 10, acetic 5)	24	++	Preserved
Sublimate-formol (sat. aq. $HgCl_2$ 85, formalin 10, H_2O 5)	24	++	Preserved
Sublimate-sulphuric (sat. aq. $HgCl_2$ 85, H_2O 10, H_2SO_4 5)	24	−	Laked
Sublimate-formol-sulphuric (sat. aq. $HgCl_2$ 85, formalin 10, H_2SO_4 5)	24	−	Slight hemolysis
Zenker-without-acetic ($HgCl_2$ 6, $K_2Cr_2O_7$ 2.5, H_2O 100 + 15)	18	++	−
Zenker-formol-hydrochloric ($HgCl_2$ 6, $K_2Cr_2O_7$ 2.5, H_2O 100, formalin 10, HCl 5)	18	+++	Subtotal hemolysis
Zenker-formol-acetic ($HgCl_2$ 6, $K_2Cr_2O_7$ 2.5, H_2O 100, formalin 10, acetic 5)	18	++	Partial hemolysis

Following these fixation experiments paraffin sections from blocks of tissue fixed in fixatixes not containing formaldehyde and showing preservation of hemosiderin were treated with acids for varying periods as in the first experiments and the results are presented in Table VII.

From this table it is evident that hemosiderin fixed with potassium bichromate and corrosive sublimate (Zenker-without-

TABLE VI

Tests of Fixing Solutions on Preservation of Hemosiderin in One Spleen

Fixing solution	Time	Amount of hemosiderin remaining	Red cells
	hrs.	± blue	
Picro-sublimate (5% $HgCl_2$ in sat. aq. picric acid)	24	+ green + brown	Preserved
Picro-sublimate-sulphuric (5% $HgCl_2$ in sat. aq. picric + 5% H_2SO_4)	24	+	Partial hemolysis
Picro-sublimate-formol (5% $HgCl_2$ in sat. aq. picric + 10% formalin)	24	+++	Preserved
Picro-sublimate-formol-sulphuric (5% $HgCl_2$ in sat. aq. picric, 10% formalin, 5% H_2SO_4)	24	+	Partial hemolysis
Bouin (sat. aq. picric 75, formalin 20, acetic 5)	24	+ brown	Partial hemolysis
Carnoy (abs. alcohol 60, $CHCl_3$ 30, glacial acetic 10)	24	++	Laked
Formol-Carnoy (abs. alcohol 60, $CHCl_3$ 30, formalin 10)	2	+ green + brown	Partial hemolysis
Carnoy-formol (abs. alcohol 60, $CHCl_3$ 25, formalin 10, glacial acetic 5)	2	−	Partial hemolysis
Absolute alcohol	18	+ green	Laked
10% formalin	48	+++	Preserved
Formol-sulphuric (10% formalin, 5% H_2SO_4)	48	++ brown	Laked

acetic) is materially less resistant to acid extraction than is formalin-fixed pigment, and that Carnoy-fixed pigment is intermediate in its resistance. The same differences in extracting power among sulphuric, formic and acetic acids as noted previously were seen in this series. Mercuric chloride, as previously indicated in the fixation experiments, apparently shows feeble extracting power, again more in the Zenker-without-acetic material than in the Carnoy-fixed specimens.

To confirm and extend these results a further series of rats

was injected subcutaneously with phenylhydrazine hydrochloride in single doses of 80 mg. per kg. and killed 4, 8, 16 and 24 hours, and 2, 3, 4 and 6 days later, and blocks of the spleens, kidneys and livers were fixed each by 7 fixation methods as follows: 85 per cent alcohol, 24 hrs.; Carnoy's fluid (absolute alcohol 6, chloroform 3, glacial acetic acid 1) 2 hrs.; corrosive sublimate (saturated $HgCl_2$ 85, H_2O 15) 24 hrs.; Zenker-without-acetic, 24 hrs.; 10

TABLE VII

Test of the Solubility of Hemosiderin in Paraffin Sections in Various Reagents at 25°C. after Fixation with Fluids not Containing Formalin

Fixation	Extracting agent	Time	Amount of pigment remaining	Color with acid ferrocyanide	Erythrocytes color with ferrocyanide
		hrs.			
Zenker-without-acetic	10% H_2SO_4	4	–	–	Greenish
		8	–	–	–
		16	–	–	Faintly greenish
		24	–	–	–
	20% formic	24	–	–	–
		48	–	–	Pale blue
	20% acetic	24	++	Blue	–
		48	++	Blue	–
	Saturated $HgCl_2$	24	+	Blue	–
		48	+	Faint blue	–
Carnoy's	10% H_2SO_4	4	±	Blue	Laked
		8	–	–	Laked
		16	–	–	Laked
		24	–	–	Laked
	20% formic	24	+	Blue-green	Laked
		48	+	Blue, green,brown	Laked
	20% acetic	24	++	Blue	Laked
		48	++	Blue	Laked
	Saturated $HgCl_2$	24	++	Blue-green	Laked
		48	++	Blue-green	Laked

per cent formalin, 48 hrs.; 10 per cent formalin and 5 per cent formic acid, 48 hrs.; and the last at 56°C. for 48 hours.

Hemosiderin was best preserved by potassium bichromate and corrosive sublimate (Zenker-without-acetic), 10 per cent formalin, and corrosive sublimate, fairly well with Carnoy's fixative, somewhat less with 85 per cent alcohol, particularly in the central portions of the blocks, and about one-third with the formic acid-formalin at room temperature. The last reagent acting at 56°C. for 48 hours not only removed all of the hemosiderin, but seriously impaired nuclear staining as well.

In passing it may be noted that appreciable quantities of iron-

reacting pigment appeared in the spleen in 16 hours and in the liver in 48. Only 1 animal, killed after 4 days, showed blue granules with ferrocyanide in the epithelium of the renal convoluted tubules.

Sections fixed in several of the foregoing solutions were further tested for the solubility of the hemosiderin in 10 per cent sulphuric acid at 25°C. and at 37°C. (Tables VIII and IX).

At 25°C. small amounts of pigment coloring blue to green with ferrocyanide were evident after 1 hour in the Carnoy and

TABLE VIII

Extraction of Pigment by 10% Sulphuric Acid at 25°C. after Various Fixatives

Fixative	No. of rat	Organ	10% H_2SO_4 at 25°C.			
			1 hr.	2 hrs.	4 hrs.	Control
Carnoy's	38 2d	Liver	–	–	–	±
		Spleen	± blue s.c.	–	–	+
	43 3d	Liver	–	–	–	++
		Spleen	± blue-green	–	–	+
$HgCl_2$	38 2d	Liver	–	–	–	±
		Spleen	–	–	–	++
	43 3d	Liver	–	–	–	++
		Spleen	–	–	–	++
Zenker-without-acetic	38 2d	Liver	± brown	± brown	–	+
		Spleen	± brown-green	–	–	++
	43 3d	Liver	± brown	–	–	++
		Spleen	± gray-brown	–	–	++
10% formalin	38 2d	Liver	–	–	–	±
		Spleen	–	–	–	++
	43 3d	Liver	–	–	–	++
		Spleen	–	–	–	++

Zenker-without-acetic material, but not later, while at 37°C. some brown iron-free pigment remained for 1 and 2 hours but no free iron remained.

Comparing Table VIII with Table II, it is seen that the pigment in the last experiment, fixed within a few days of its appearance, was more readily removed by sulphuric acid than was the probably older pigment in the human material.

Following this indication that recently formed hemosiderin might be more readily soluble in acids than older pigment, further experiments with phenylhydrazine * were carried out.

* Mice averaging 21 gm. were used. A dose of 5 mg. (240 mg. per kg.) of phenylhydrazine hydrochloride killed 92 per cent of 25 mice in less than 24 hours, most of them in 30 minutes. Doses of 2 and 3 mg. (95 and 145 mg. per kg.) were

With phenylhydrazine, traces of iron-containing pigment appear in the spleen in 24 hours. Very considerable amounts are evident in the splenic follicles in 48 hours, less in the pulp. The intrafollicular pigment persists only for 5–6 days, and reacts with ferrocyanide throughout that period. Considerable amounts of brown pigment which fail to react with ferrocyanide persist in the pulp and less in the follicles for as long as 25 days. This pigment also fails to react with potassium ferricyanide.

On testing sections of those spleens showing considerable amounts of iron-reacting pigment for solubility of the pigment

TABLE IX

Extraction of Pigment by 10% Sulphuric Acid at 37°C. after Various Fixatives

Fixative	No. of rat	Organ	10% H_2SO_4 at 37°C.				
			1 hr.	2 hrs.	3 hrs.	5 hrs.	Control
Carnoy's	272 4d	Liver	−		−	−	++
		Spleen		−	−	−	+
	271 6d	Liver	−	−	−		++
		Spleen	−	−	−	−	++
$HgCl_2$	272 4d	Liver	−	−			++
		Spleen		−	−	−	++
	271 6d	Liver			−	−	±
		Spleen	−		−	−	++
Zenker-without-acetic	272 4d	Liver	± brown	± brown			±±
		Spleen		−	−	−	++
	271 6d	Liver		± brown	−	−	++
		Spleen	++ brown		−	−	++
10% formalin	272 4d	Liver	++ brown	++ brown		−	++
		Spleen		−	−	−	++
	271 6d	Liver		−	−	−	++
		Spleen	++ brown		+ brown	−	++

in 10 per cent sulphuric acid at room temperature, it was found that in all animals the intrafollicular iron-containing pigment was removed in one-half to 1 hour, or perhaps persisted for some hours as an iron-free brown pigment. In any case, no iron reaction was positive after more than 1 hour in acid.

As iron-containing pigment disappeared in this series in a short period no indication was given as to alteration of acid solubility of hemosiderin with age.

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better tolerated, 92 and 78 per cent respectively surviving for 24 hours, and 84 and 62 per cent for 48.

A series of 7 formalin-fixed spleens containing considerable quantities of hemosiderin in the pulp, from animals intoxicated with organic selenium compounds for periods varying from 25 to 380 days were studied in respect to the acid solubility of the pigment. The exposures to organic selenium were 25, 32, 156, 342, 372, 373 and 380 days, the time necessary for solution of the iron-containing pigment in 10 per cent sulphuric acid at room temperature was 2, 2, 2, 8, 2, 8 and 4 hours.

A series of 24 formalin-fixed spleens from rats intoxicated with sodium vanadate for periods varying from 2 to 53 days were tested as to the solubility of the pigment in 10 per cent sulphuric acid at room temperature. Regardless of the length of exposure to vanadium, the pigment disappeared in approximately 4 hours in all animals.

Apparently the age of the pigment is not a determining factor in its resistance to solution in 10 per cent sulphuric acid.

From the foregoing, it was noted that the iron of hemosiderin is quickly soluble in oxalic acid and very slowly if at all soluble in acetic acid. Standard references on solubilities of inorganic salts note that ferric oxalate is highly soluble in water and basic ferric acetate insoluble, while ferrous oxalate is insoluble in water and ferrous acetate quite soluble. This would appear to indicate that the iron content of hemosiderin is largely ferric.

In studies on the toxicology of sodium formaldehyde sulphoxylate done in 1933, I found that none of the copious iron-containing pigment reacted directly with potassium ferricyanide. Gömöri [17] also noted that he has never obtained a direct reaction with ferricyanide and concludes that hemosiderin does not contain ferrous compounds thus demonstrable.

Having noted that ferrous sulphate in solution is almost immediately converted to the ferric form by hydrogen peroxide, I decided to try various oxidizing and reducing agents on heavily hemosiderotic lung, liver and spleen, considering that the first would theoretically contain the least ferrous iron, and the two latter more, if any such were to be found. The results are presented in Table X.

From this table it is noted that there is no evident increase in demonstrable ferric iron on oxidation with H_2O_2, that no ferrous iron is demonstrable with ferricyanide, even after reduction with

TABLE X

The Effect of Oxidizing and Reducing Agents on the Reactions of Hemosiderin after Formalin Fixation with Potassium Ferrocyanide and with Potassium Ferricyanide

Preliminary treatment	Results with ferrocyanide			Results with ferricyanide		
	Amount	Form	Color	Amount	Form	Color
None	+++	Granular	Dark blue	+++	Granular	Brown
Hydrogen peroxide USP 1 hr. 25°C.	+++	Granular	Dark blue	+++	Granular	Brown
4% elon * 1 hr. 25°C.	+++	Granular	Dark blue	+++	Granular	Brown
4% hydroquinone 1 hr. 25°C.	+++	Granular	Dark blue	+++	Granular	Brown
10% pyrogallic in half saturated sodium carbonate 1 hr. 25°C.	+++	Granular	Dark blue	+++	Granular	Brown
10% sodium sulphite 1 hr. 25°C.	+++	Granular	Dark blue	+++	Granular	Brown
Yellow ammonium sulphide sol. in 3 parts alcohol 17 hrs. 25°C.	++	Granular	+ blue halos	++	Granular	+ blue halos

* Elon = trade name for monomethylparamidophenol sulphate, a photographic developer.

elon, hydroquinone, alkaline pyrogallol or sodium sulphite, and that after treatment with ammonium sulphide part of the iron still reacts with ferrocyanide, and part with ferricyanide. The quantity in both cases is evidently less than with the direct ferrocyanide procedure or with the Quincke iron sulphide reaction, and marked diffusion of the blue stain out from the granules is noted.

This agrees completely with Gömöri's [17] findings and further indicates that, as part of the iron still reacts as ferric after the ammonium sulphide treatment, the Tirmann-Schmelzer sulphide Turnbull blue reaction affords an incomplete and partial demonstration of the iron content of the pigment.

Discussion

Hemosiderin after fixation with formalin is rather slowly soluble in dilute acids, best in oxalic, then sulphuric, then nitric, formic and aqueous hydrochloric acids. It is very slowly soluble in hydrochloric acid alcohol and almost insoluble in 20 per cent acetic acid.

Higher concentrations of acid increase the speed of solution, and elevation of temperature facilitates solution of at least the iron component of hemosiderin.

Fixatives that contain acids but no formalin often completely remove hemosiderin or alter it so that brown pigment containing no demonstrable iron is produced. The presence of formalin in the fixative often protects hemosiderin against acetic, formic and hydrochloric acids, but not sulphuric. Alcohol is inferior to formalin as a fixative for hemosiderin.

Formalin in the fixing fluid tends to make hemosiderin more resistant to subsequent extraction with acids.

The alteration of the solubility of the iron in hemosiderin by certain fixatives as compared with others and with unfixed material indicates that iron exists in a protein combination. This concurs with the views of Hueck,[5] Schmidt,[18] Neumann,[19] and Arnold.[20]

That brown granular pigment containing no demonstrable iron may persist in areas of old hemorrhage after the disappearance of hemosiderin has often been noted. The present findings show that a similar pigment may be produced from hemosiderin by

the presence of acids in the fixing fluids, or by the action of warm acids on previously fixed material. This indicates that hemofuscin may be derived from hemosiderin by the loss of its iron content.

It seems clearly indicated that the iron of hemosiderin is in the ferric state. Even powerful reducing agents fail to reduce it to the ferrous form, and even after conversion into iron sulphide, part of the iron appears to remain in, or very promptly to revert to, the ferric state.

Considerable variation in the speed of solution of hemosiderin in acids is noted from case to case, and in experimental hemosiderosis with the mode of its production. The age of the pigment appears to have little influence on its speed of solution. Granule size has a material influence on speed of solution, coarser granules resisting longer.

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THE POLARIZED LIGHT METHOD FOR THE STUDY OF MYELIN DEGENERATION AS COMPARED WITH THE MARCHI AND SUDAN III METHODS *

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Introduction

For many years the methods most commonly used for the demonstration of myelin degeneration in the peripheral and central nervous system have been the Weigert, the Marchi, the sudan III, and the scarlet red.

The use of the Marchi method has been criticized by numerous authors because of its capriciousness and because of the large amount of artefact frequently present which interferes considerably with correct diagnosis in cases of suspected early degenerative change. The Weigert, the sudan III and scarlet red methods, although apparently giving consistent results, have the disadvantage of not demonstrating the early phases of myelin change.

In connection with the study of vitamin B_1 deficiency in the rat, we have had occasion to examine the nervous tissues of approximately 500 animals. In these studies the variability of the Marchi method, and the unsuitability of the sudan III and scarlet red methods for the detection of early myelin change in the nerves of rats were thoroughly confirmed.

The results obtained by Sutton, Setterfield and co-workers [1–4] with polarized light in the study of early myelin changes in degenerating nerves indicated that it might be advantageously used in the study of nerves from vitamin B_1 deficient animals. As the method had not been generally used it seemed desirable to subject it to a critical study in which both normal and transected nerves would be examined by the polarized light method as well as by the conventional Marchi and sudan III methods. The results confirm and extend the findings of Sutton and his co-workers, and it is believed that the further observations made are fundamental and

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will be of value to anyone wishing to use the method in similar studies.

Experimental

The nerves for this study were obtained from normal rats 6 to 8 weeks of age and approximately 150 gm. in weight. They had been fed the complete ration used in the stock colony.[5] The right and left lumbosacral and sciatic nerve trunks from 142 rats were studied. Of these, 71 rats were used for studies of the normal nerve, 50 of which were studied by the polarized light method, 11 by the Marchi method, and 10 by the sudan III method. In the remaining 71 rats the right nerves were transected while the left nerves were left intact and studied as normal controls; in this group 28 were studied by the polarized light method, 23 by the Marchi, and 20 by the sudan III method.

For the study of progressive degenerative changes due to transection of the nerve, the animals were lightly anesthetized with ether, the sciatic trunk was cut as high up in the leg as possible, and about 1 mm. of the nerve was removed. When the desired length of time after transection had elapsed, these animals were killed by decapitation. The progress of degenerative change was studied at intervals of 24, 48, 72, 120 and 216 hours after transection. There was no evidence of infection during the interval between transection and killing. The animals used for studies of the normal nerve were either decapitated or killed with chloroform.

After the skin was removed from over the posterior extremities, the muscles were cut away to bare the lumbosacral and sciatic trunks of the right and left extremities so that rapid fixation would take place. As it was considered undesirable to remove the nerves from the legs before fixation, the entire pelvis and extremities were placed in the desired fixative.

The tissues used for the polarized light and sudan III methods were fixed in 10 per cent neutral formalin (Merck) for 48 hours, the fixative being changed at the end of 24 hours. The nerves were removed from the extremities at the end of 48 hours and stored in fresh 10 per cent neutral formalin until sectioned.

The tissues used for the Marchi method were fixed in Müller's fluid for 7 days, the fixative being changed daily for the first 3 days. The nerves were removed from the legs at the end of the 2nd day. After fixation the nerves were placed in Marchi's fluid for 7 days,

washed, dehydrated, and embedded in pyroxylin as rapidly as possible.*

Before the nerves were sectioned they were arbitrarily divided into three pieces: the "proximal" portion consisted of the lumbosacral trunk from its exit from the vertebral column to the head of the femur; the "medial" portion consisted of the sciatic trunk from the head of the femur to the knee joint; and the "distal" portion extended from this point to the heel. In transected nerves only the portions distal to the cut were studied. These corresponded to the "medial" and "distal" portions of the normal nerve.

For study by the polarized light method, frozen sections were made on a clinical microtome at 10, 20 and 35 microns. The sections were cut into distilled water and immediately mounted in glycerin or glycerin jelly.[6] Because of the clearing action of the mounting medium, visual observations were made about 30 minutes after mounting in order to secure as constant a picture as possible. With only a few differences, which will be discussed later in this paper, the technic of visual observation was essentially the same as that described by Sutton, Setterfield and Krauss.[1] As the mounts were not permanent, microphotographs were taken within 60 minutes after mounting and constituted the only permanent record of the section. The microphotographs were taken at 500 diameters at the point of greatest birefringence between crossed Nicols.

For study by the sudan III method, frozen sections were made at 20 microns,* stained, and mounted in glycerin jelly. The nerves prepared by the Marchi method were cut at 20 microns,† dehydrated, cleared in xylol and mounted in dammar.

Results

The Polarized Light Method

General Observations: During the early part of this study it was noted that when normal nerves were cut at 20 microns, numerous

* Comparison of frozen sections of unembedded nerves prepared by the Marchi method with those embedded in pyroxylin indicated that this rapid dehydration and embedding did not alter the resulting picture.

† Thinner sections (10 microns) were studied, but in this case the thickness of sections showed no apparent differences or advantages, and sections cut at 20 microns were used throughout this study.

fibers at the periphery and occasionally at the ends showed marked differences in structure from those in the body of the section. There was considerable darkening of the fibers as compared with the body of the section, and the proportion of the myelin sheath to the axis cylinder was greater. Nodes of Ranvier and incisures of Schmidt-Lantermann were also more frequent and more clearly seen. It was also observed that the same structure was exhibited by isolated fibers, the more distal portions of the nerve trunk, and occasional sections which were thinner than usual. It seemed apparent that the thickness of the section had some bearing on these differences, and a study of the effect of thickness on the details of structure was made.

In order to determine the effect of the thickness of the sections on the visibility of structural details, a series of sections 10, 20 and 35 microns in thickness was prepared. Figures 3 to 5 illustrate the results of this study in normal nerves, and Figures 6 to 8 show the effect of thickness in transected nerves. The structure of the fibers in sections cut at 10 microns (Fig. 1) more closely resembled the structure of isolated fibers (Fig. 1) than those in sections cut at 20 or 35 microns (Figs. 2 and 3). The sections cut at 10 microns constituted roughly a single layer of fibers, and the amount of birefringent material was reduced to a minimum. Visibility was thus increased and fibrillar and interfibrillar structures were more clearly depicted. The increase in birefringent material (myelin sheaths) in the thicker sections caused a haze, which almost completely obliterated any details of structure in the sections cut at 35 microns. It is interesting to note in this connection that in taking microphotographs of sections of different thickness, about one-fifth the exposure necessary for the sections cut at 10 microns was adequate for those cut at 35. Variation in the size of individual fibers within a given section could be clearly seen in the thinner sections (10 microns), whereas in the thicker ones (20 and 35 microns) the haziness often obliterated them altogether.

After considerable study of sections of different thickness, the sections cut at 10 microns were selected as offering the truer representation of the individual fibers and their relations to each other. This thickness was used throughout the remainder of the study.

Since it was desired to apply this method to the study of the peripheral nerves of vitamin B_1 deficient animals where autopsy

material was desired, a study was made to determine the effect of decapitation as compared with killing with chloroform. No apparent differences due to the method of killing were noted in any of the nerves studied.

In variation from the method of Sutton, Setterfield and Krauss,[1] some of the sections were mounted in glycerin jelly [6] rather than in glycerin. This semisolid medium allowed the sections to be safely photographed with the microscope in the horizontal position; it also had the advantage of making it possible to weight the coverglass, and as the material hardened the section remained flattened. A further advantage was that the nerve did not clear so rapidly as when mounted in glycerin. A comparison of sections mounted in both mediums showed that although the glycerin jelly gave a somewhat darker preparation, this did not interfere in any way with the study of the nerve.

The Normal Nerve and its Variations: In 10 micron sections the individual fibers of the nerve did not always follow the parallel course seen in preparations cut at 20 microns. However, the slight waviness seen at times would hardly be mistaken for the tortuosity present in degenerating fibers. Individual fibers were quite regular in size along their course except at the nodes of Ranvier, but differed in size as compared with other fibers in the same area. The fibers seen in the "medial" and "distal" portions of the nerve were usually smaller in diameter, and the proportion of the myelin sheath to the axis cylinder was usually less, although more variable, than in the "proximal" portion.

The myelin sheath was usually birefringent, brilliantly clear at the points of greatest birefringence, and rarely contained isotropic materials. All the materials in the sheath, however, did not become dark at the points of extinction; this gave the section a finely granular appearance. The nodes of Ranvier varied considerably in size and in structure, and were at times very granular. Part of the granulations remained light at the points of extinction. In the greater number of cases, however, the nodes showed club-shaped enlargements which were similar to those previously found in Busch preparations.[5] The incisures of Schmidt-Lantermann were not always present but were quite frequently seen. When visible, they were irregularly placed, their apices varied in direction, and they contained variable amounts of isotropic material. Although

they had been observed at all thicknesses, they were more frequently seen in the 10 micron (Fig. 1) than in the thicker sections (Figs. 2 and 3). The axis cylinders were isotropic and varied somewhat in size. Their borders were usually sharply defined, but at times were mildly roughened. Tortuosity of the axis cylinder was not observed in normal fibers. Interfibrillar connective tissue was isotropic, as were perineural fat deposits. The interrelationship of fibers could often be seen better by uncrossing the Nicol prisms and cutting down the light.

The Transected Nerve: The earliest period at which degenerative change was studied in these experiments was 24 hours after transection, at which time marked and consistent changes were observed. Although the severity of the degenerative change observed at any given interval after transection varied somewhat in different nerves, the progress of the degeneration after 24 hours was essentially as shown by Sutton, *et al.*,[1] and will not be repeated here. However, in the transected nerve as well as in the normal, thickness of the section was an important consideration. Although the edema consistently present in the transected nerves tended to separate the fibers to a variable extent and thus show more isolated fibers than in normal nerves, the thicker sections were hazy and details of structure were at times markedly obscured (Figs. 4–6).

In the earlier stages of the degenerative change localized areas of swelling and apparent fragmentation of the fibers were noted which did not become blackened at the points of extinction. Either this material was not birefringent, or else it was composed of numerous droplets of still normal myelin which did not allow rotation of the section to cause extinction because of its multiple refractive surfaces.

It was often of very great advantage when confronted with hazy undifferentiated threads of fibrillar tissue which seemed partially isotropic, or with a black space between the nerve fibers, to uncross the analyzer prism and cut down the light by lowering the substage condenser. By this means one could distinguish between a tangentially cut fiber which was discontinuous and showed its thready nature, and an isotropic portion of a fiber which usually showed the sheath elements and its continuity with the birefringent portions of the fiber. It could be determined in the same manner

whether a black structureless space between fibers was a split in the section or a darkened area composed of completely isotropic fibers (Figs. 16–19).

The above procedure was also of value in ascertaining whether the axis cylinder of a nerve fiber was swollen or whether there was periaxillar accumulation of isotropic material. In some cases fusiform areas of blackening were seen along the course of the nerve fibers. By uncrossing the Nicols one could often determine whether this represented an accumulation of degenerated myelin or a Schwann cell.

During the progress of the degenerative change many fibers were seen which assumed a "sausage-link" structure, apparently indicating fragmentation. However, when such fibers were viewed with the analyzer uncrossed numbers of them were found which were still contained within an intact sheath (Figs. 16 and 17).

The nerves of 8 animals (16 sciatics) were studied in the fresh state without previous fixation. Frozen sections were made 10 or 20 microns in thickness and mounted in glycerin. The sections were cut into distilled water but remained in it only a few seconds before being placed on a slide and mounted. Study of these nerves revealed a completely different picture from that observed in the fixed nerves (Figs. 13–15). The fibrillar structure was not clearly seen and the tissue reacted only minimally or not at all to rotation of the stage. Degenerated nerves showed more similarity to the fixed nerves than did the normal nerves. A comparison of sections cut at 10 and 20 microns showed that, as in the case of fixed nerves, the increase in myelin caused considerable distortion (Figs. 13 and 14).

The Marchi and the Sudan III Methods

Normal nerves prepared by the Marchi technic exhibited a marked variation in the type and amount of blackened material present. This variability was so great that correct diagnosis was at best exceedingly difficult and uncertain. Nerves transected for 96 hours were seen which showed very little staining with osmic acid, while normal nerves frequently contained copious amounts of blackened droplets. In the transected nerve the first stage at which degenerative change could be definitely ascertained was 72 hours after transection (Fig. 9). The amount of blackened

material indicative of degeneration present at any given interval varied more than was indicated in comparable preparations studied by the polarized light method. Segmentation and fragmentation could be seen clearly as the light was decreased by lowering the substage condenser in areas that showed no blackening with osmic acid. Sections prepared by the Marchi and polarized light methods at intervals of 72 and 120 hours after transection are compared in Figures 9–12. In contrast to the polarized light method only myelin changes are seen in the Marchi preparations.

The normal nerves prepared by the sudan III method showed no evidence of staining except for perineural fat deposits which were intensely dyed. The earliest definite staining of degenerated fibers in transected nerves was 120 hours after transection, although segmentation and other fibrillar changes could be seen clearly long before this time despite the lack of staining.

Discussion

The results obtained in studies of normal and degenerated nerves by the polarized light method show that it is a valuable supplement to the older methods for the demonstration of myelin change. Its chief disadvantages are the comparatively small amount of critical study to which it has been subjected and the necessity of taking routine microphotographs in order to obtain permanent records. As with all new technics, this method demands that careful and critical observations be made until one is thoroughly familiar with its intricacies. On the other hand, it has many advantages over the older methods. It is simple, rapid and consistent. Technical manipulations are reduced to a minimum, thus lessening the possibility of producing artefacts. Both axis cylinder and myelin sheath changes are shown in the same preparation. Moreover, myelin sheath changes after transection of nerves can be demonstrated much earlier by the polarized light method than by either the Marchi or sudan III methods.

The thickness of the section is very important in the polarized light method. It is believed that sections cut at 10 microns offer a truer representation of the structure of the individual fibers and their interrelations than do those cut at 20 or 35 microns. Since those cut at 10 microns represent roughly a single layer of fibers, the haziness due to the birefringent material is minimal and the

details are more clearly seen than in the thicker sections. The use of a microtome which is more accurate than the clinical microtome would be advantageous.

Although only a minimal amount of evidence is at hand, it indicates that the structures observed in normal and degenerated nerves by the polarized light method are to some extent the result of fixation in formalin. The myelin sheaths of fresh (unfixed) nerves exhibited only slightly the property of birefringence, although in two animals, whose sciatic nerves had been transected for 216 hours, the typical "Maltese cross" effect (Fig. 15) consistently seen in degenerated nerves fixed in formalin was visible but reacted only slightly to rotation of the stage. This effect of formalin, however, does not detract from the usefulness of the method.

Summary

1. A study has been made of the merits of the polarized light method as compared with the Marchi and sudan III methods in demonstrating myelin degeneration due to transection of the peripheral nerves of the rat.

2. The polarized light method was found to be rapid and accurate. The changes depicted were consistent and did not depend on numerous technical manipulations for their demonstration. Both myelin sheath and axis cylinder changes were visible in the same preparation.

3. As compared with the polarized light method, the Marchi method gave very inconsistent results. The sudan III method was consistent but failed to reveal the early changes following transection.

4. Marked and advanced changes were shown by the polarized light method in nerves which had been transected only 24 hours. The earliest degeneration shown by the Marchi method was 72 hours after transection and by the sudan III method 120 hours after transection.

5. The thickness of the section influenced the structural detail observed by the polarized light method. Sections 10 microns in thickness showed more detail than thicker sections.

6. It was found to be advantageous to uncross the analyzer prism for determining the continuity of fibers which appear seg-

mented, for distinguishing between edema of the axis cylinders and periaxillar accumulation of isotropic material, and for revealing the presence of isotropic fibers masked by the crossed Nicols.

7. Sections of fresh unfixed normal or degenerating nerves, when viewed by polarized light, presented an appearance considerably different from that of fixed nerves. This does not detract, however, from the usefulness or reliability of the method.

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DESCRIPTION OF PLATES

PLATE 42

All microphotographs were taken at a magnification of 500 diameters. Except for Figs. 9, 11, 17 and 19, all microphotographs were taken in polarized light at the point of greatest birefringence between crossed Nicols.

Wratten metallographic plates were used with a contrast developer (Eastman D–19) for photographs of preparations studied by the polarized light method. Wratten "M" plates were used for the photographs of the Marchi preparations.

FIG. 1. Normal nerve. Microphotograph of the "proximal" portion showing structural details as seen in a section cut at 10 microns. Note the fibrillar detail, the proportion of myelin sheath to axis cylinder, the presence of numerous incisures of Schmidt-Lantermann, and the nodes of Ranvier. Compare with Figs. 2 and 3. Polarized light method.

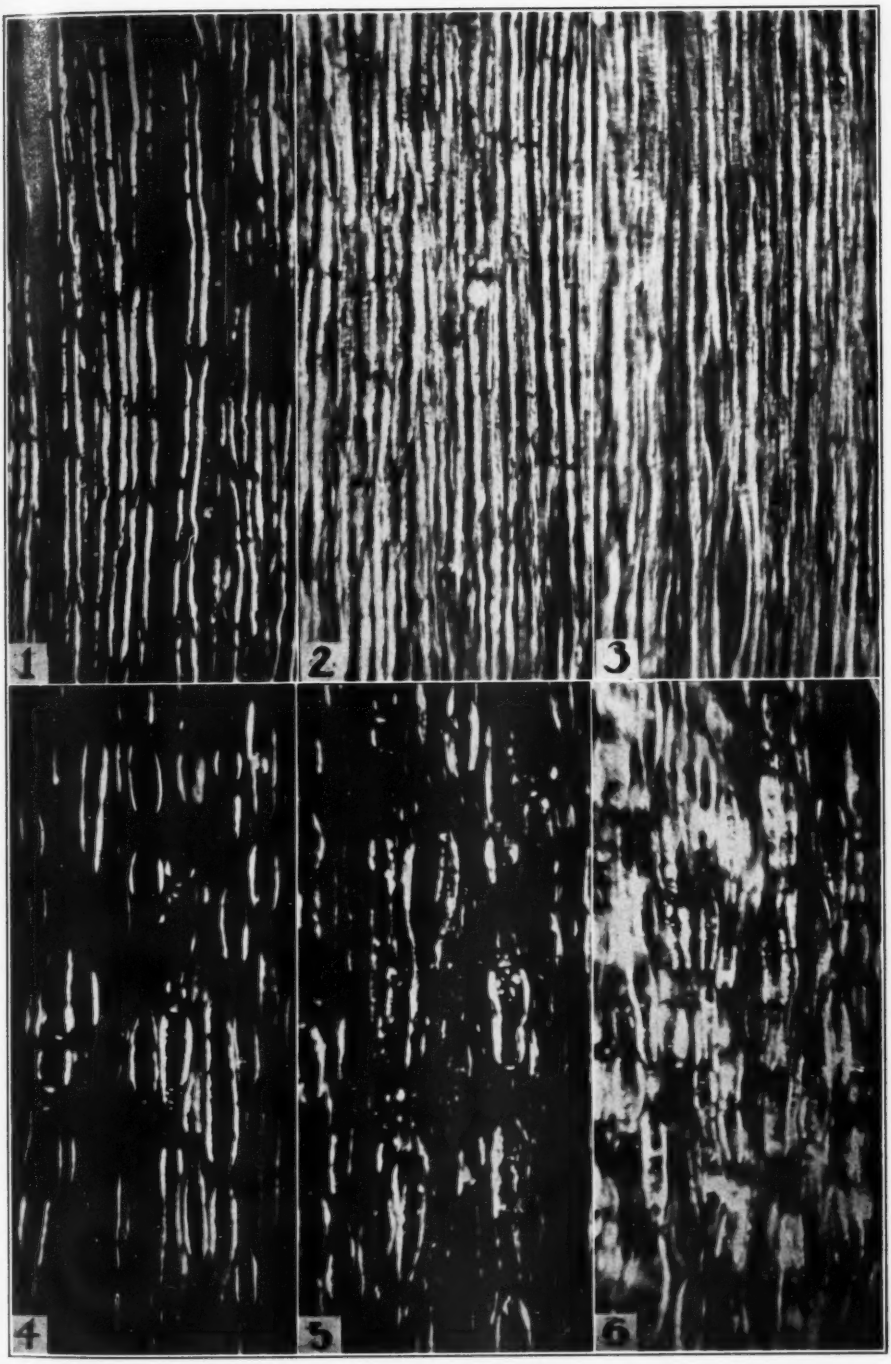
FIG. 2. Normal nerve. Microphotograph of the same portion of the same nerve shown in Fig. 1. Note the increase in haziness and loss of detail in sections cut at 20 microns. Polarized light method.

FIG. 3. Normal nerve. Microphotograph of the same portion of the same nerve as shown in Figs. 1 and 2. Note the almost complete loss of detail in sections cut at 35 microns. Compare the structure of the node of Ranvier in the middle right hand portion of the photograph with the nodes in Fig. 1. Polarized light method.

FIG. 4. Degenerated nerve. Microphotograph of a section of a nerve which had been transected for 48 hours. Edema, segmentation and increase in isotropic material in the individual fibers are clearly seen. Section cut at 10 microns. Compare with Fig. 1. Polarized light method.

FIG. 5. Degenerated nerve. Microphotograph of the same portion of the same nerve as shown in Fig. 4. Note the loss of detail and interference due to superimposed layers of fibers in sections cut at 20 microns. Polarized light method.

FIG. 6. Degenerated nerve. Microphotograph of the same portion of the same nerve shown in Figs. 4 and 5. Note increased interference and haziness in section cut at 35 microns. Polarized light method.



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Polarized Light for Study of Myelin Degeneration

PLATE 43

FIG. 7. Normal nerve. Microphotograph of the "medial" portion of the same nerve shown in Figs. 1, 2 and 3. Note the variations in size of the fibers and the proportion of myelin to axis cylinder. Compare with Figs. 1 and 8. Polarized light method. Section cut at 10 microns.

FIG. 8. Normal nerve. Microphotograph of "distal" portion of the same nerve shown in Figs. 1, 2, 3 and 4. Note the decrease in size of the fibers to the axis cylinders as compared with "proximal" and "medial" portions. Polarized light method. Section cut at 10 microns.

FIG. 9. Degenerated nerve. Microphotograph of a nerve which had been transected 72 hours showing the irregular black precipitation seen in Marchi preparations. Compare with Fig. 10. Marchi method. Section cut at 20 microns.

FIG. 10. Degenerated nerve. Microphotograph of a nerve that had been transected for 72 hours as shown by the polarized light method. Note the clear sheath and axis cylinder changes that are shown. Compare with Fig. 9. Polarized light method. Section cut at 10 microns.

FIG. 11. Degenerated nerve. Microphotograph of a nerve that had been transected 120 hours. Marchi method. Section cut at 20 microns.

FIG. 12. Degenerated nerve. Microphotograph of a nerve that had been transected 120 hours. Polarized light method. Section cut at 10 microns.

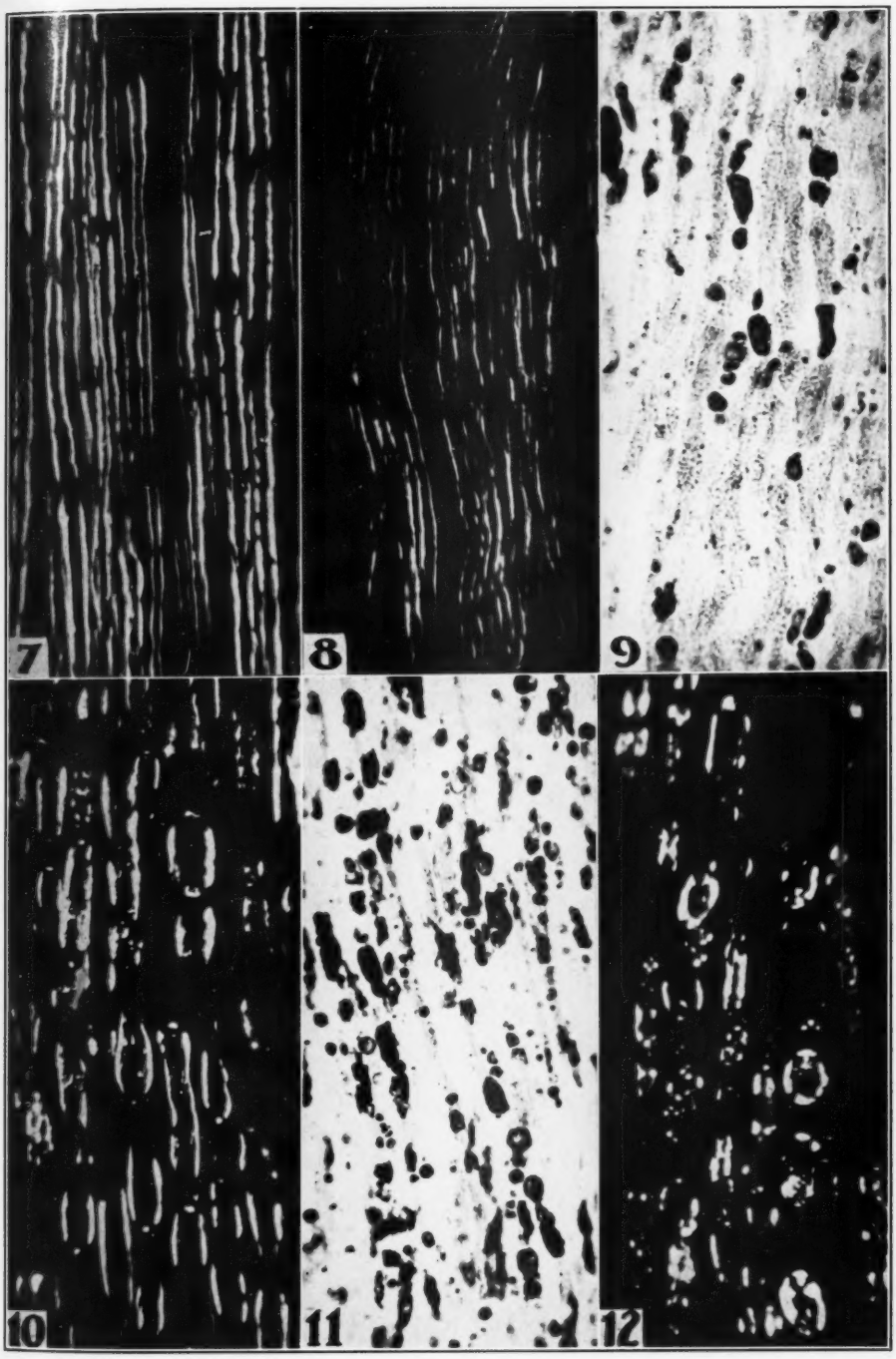

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PLATE 44

FIG. 13. Normal nerve. Microphotograph of a fresh (unfixed) nerve showing the lack of differentiation and structural detail as compared with the fixed nerve shown in Fig. 1. Such preparations react only minimally to rotation of the stage. Polarized light method. Section cut at 10 microns.

FIG. 14. Normal nerve. Microphotograph of a section cut at 20 microns of the same portion of the same nerve shown in Fig. 13. Note the increase in birefringent material as compared to Fig. 13 and the lack of typical fibrillar detail as shown by fixed nerves. Polarized light method.

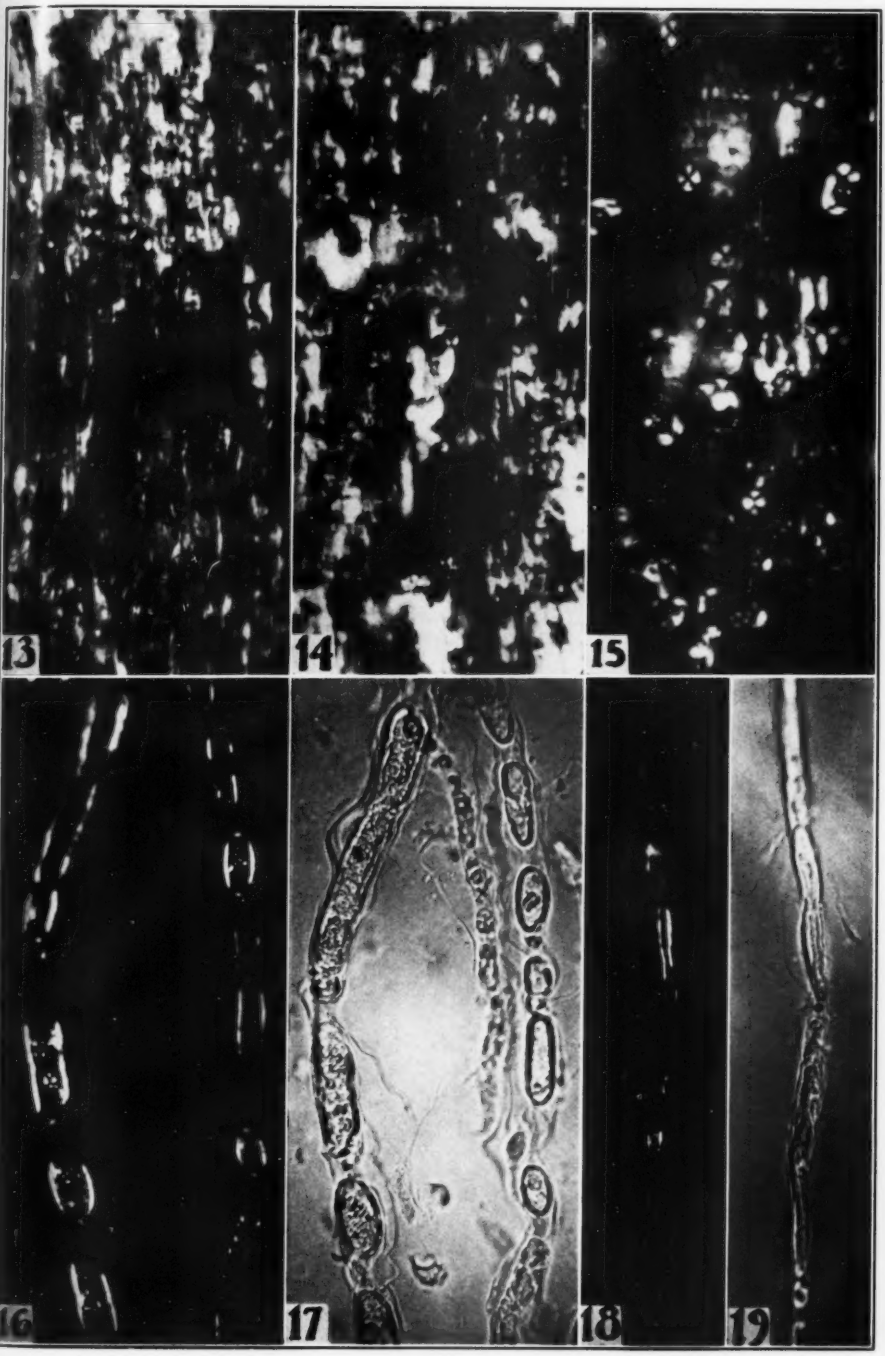
FIG. 15. Degenerated nerve. Microphotograph of a section of a fresh (unfixed) nerve that had been transected for 96 hours. The similarity between the fixed and unfixed nerve is greater than in the normal nerve. Such sections react slightly to rotation of the stage, but structural detail is much less clear and definite than in fixed nerves. Polarized light method. Section cut at 10 microns.

FIG. 16. Degenerated nerve. Microphotograph of isolated fibers from a nerve that had been transected 72 hours. Note the segmentation and the completely isotropic areas between the segments and between the two fibers shown. Compare with Fig. 17. Polarized light method. Section cut at 10 microns.

FIG. 17. Degenerated nerve. Microphotograph of the same portion of the same nerve shown in Fig. 16, showing the advantages of uncrossing the Nicol prisms to determine whether segmentation seen with crossed Nicols is indicative of fragmentation, and also to determine whether a blackened area is a split between fibers or an area of degenerated material that has become completely isotropic. Note the continuity of the sheaths of both fibers and the appearance under these circumstances of an isotropic fiber that had been "masked" by the crossed Nicols. The wavy material between the fibers represents interfibrillar connective tissue. Section cut at 10 microns.

FIG. 18. Degenerated nerve. Microphotograph of an isolated fiber from the same nerve shown in Figs. 16 and 17. Note the difference in size of the fibers in these sections. Compare with Fig. 19. Polarized light method. Section cut at 10 microns.

FIG. 19. Degenerated nerve. Microphotograph of the same portion of the same nerve shown in Fig. 18 with the Nicol prisms uncrossed. Note that the smaller size of the fiber is to a large extent the result of perifibrillar increase in isotropic material. Without examination with uncrossed Nicol prisms this fiber would have been considered merely a smaller or a less edematous fiber than those shown in Fig. 16. Section cut at 10 microns.



Prickett and Stevens Polarized Light for Study of Myelin Degeneration

HISTOPATHOLOGY OF THE PERIPHERAL NERVES IN ACUTE AND CHRONIC VITAMIN B_1 DEFICIENCY IN THE RAT *

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INTRODUCTION

Some investigators [1-3] have reported that vitamin B_1 deficiency produces myelin degeneration in the peripheral nerves; others [4-8] have failed to find evidence of such changes. A further study was undertaken, therefore, in an effort to find some explanation of the disagreement in results. The study was planned to compare the effects of different diets and different levels of feeding as well as the effect of an acute deficiency with that of a chronic deficiency.

The present paper is concerned only with the results of the studies on the peripheral nerves. The polarized light method was the principal method used, since it has been shown [9, 10] to be more sensitive and more reliable for the detection of early myelin changes than the Marchi or the sudan III methods. The study included, however, observations of preparations made by the use of the sudan III method.

EXPERIMENTAL

Histological Technic

The histological technic was essentially the same as that described previously.[10] The rats were killed with chloroform and the posterior extremities were fixed in 10 per cent neutral formalin before removal of the nerves. The sciatic nerves were then removed from the legs and stored in fresh fixative until sectioned. Frozen sections 10 microns in thickness were used for both the polarized light and the sudan III methods.

The Acute Deficiency

Animals and Dicts: This group consisted of 133 animals, 39 of which received the stock diet [4] as a control diet, which was known to be adequate for both growth and reproduction. The diets and

* Received for publication December 12, 1938.

levels of feeding have been discussed in a previous report.[11] Animals receiving the various types of diets were used, but since the type of diet had no apparent effect on the condition of the nerves, it will be disregarded in this report. The level of feeding, however, did affect the condition of the nerves as will be shown later. There were 63 animals studied from the limited group (food limited to a maximum of 12 calories per rat daily and isocalorically controlled throughout all deficient and control diets); 44 of these received the vitamin B_1 deficient diets, 10 received the deficient diets plus vitamin B_1,* and 9 received the stock diet. There were 70 animals studied from the *ad libitum* group; 29 of these received the deficient diets, 11 received the deficient diets plus vitamin B_1,* and 30 received the stock diet.

With the exception of 8 animals used to study the effect of a vitamin B_1 deficient diet on the nerves of animals becoming moribund before developing the neuromuscular symptoms, all the deficient animals were allowed to develop severe neuromuscular symptoms before being killed. The average length of time the animals were left on experiment after developing neuromuscular symptoms was 2.5 days. No postmortem material was used for histopathological studies.

Gross Pathology: At autopsy the peripheral nerves of animals in the deficient and control groups presented no abnormalities visible in the gross.

Microscopic Pathology: The peripheral nerves of the deficient animals, when examined by the polarized light method, showed a variable degree of edematous change that did not appear to be related to the diet, the level of feeding, the time on experiment, or the intensity of the symptoms (Fig. 2). In no case did the edema approach a stage comparable to that seen in nerves transected for 24 hours.[9, 10] All fibers appeared involved and both the myelin sheath and the axis cylinder shared in the enlargement. At times the margins of the axis cylinders were somewhat roughened and the incisures of Schmidt-Lantermann contained more isotropic material than is normally seen. Occasional droplets of isotropic material were found in the myelin sheaths, but these were comparatively infrequent. The nodes of Ranvier were occasionally enlarged and dumb-bell shaped. Sudan III preparations showed

* Fuller's earth absorbate.[11]

no evidence of this edema or of any degenerative change. Perineural fat, however, was intensely stained.

The peripheral nerves of the control animals that received vitamin B_1 and the limited (isocaloric) amounts of food were significantly different from those of the deficient animals (Fig. 3). In the animals receiving the stock diet, as well as those receiving the experimental diets, a considerable number of the fibers showed changes typical of advanced Wallerian degeneration; the animals that received the stock diet showed the greatest number of degenerating fibers. The nerves of animals receiving the high carbohydrate diets plus vitamin B_1 showed slightly more degeneration than those from animals on the 23 per cent coconut fat diets. The degenerative change apparently was not confined to patchy areas but was seen anywhere in the sections and usually extended throughout the fiber. In addition the edematous changes observed in the deficient group were present in the greater number of animals.

The nerves of the control animals that received vitamin B_1 and food *ad libitum* showed no evidence of degenerative changes in amounts greater than normally present.[12]

In contrast to the above are the nerves of 8 animals in the deficient groups which had shown severe anorexia and progressive weakness and which finally became moribund without developing neuromuscular symptoms. These animals showed more Wallerian degeneration than is normally seen, but less than the controls on the limited (isocaloric) feeding schedule. The edematous changes seen in other rats in the deficient groups as well as in the control groups were also present to a variable extent. Sudan III preparations showed the Wallerian degeneration but no other evidence of abnormality.

The Chronic Deficiency

Animals and Diets: This group consisted of 40 rats, all of which received diets deficient in vitamin B_1. The diets were essentially similar to those used in the study of the acute deficiency.

The animals in this group were first allowed to develop the severe neuromuscular symptoms characteristic of the acute deficiency and were then fed small amounts of thiamin crystals dissolved in distilled water. Administration was by means of a

catheter into the stomach. The dosage was kept as small as possible to prevent complete relief of symptoms, but large enough to keep the animals alive. The dosage varied from 1.5 gamma to 100 gamma depending on the severity of the condition at the time of administration. Usually 1.5 gamma per rat daily was sufficient. The rats in this group were continued on experiment from 3 to 99 days after the development of the initial attack. However, it was not always possible to prevent cures, nor was it possible to prevent deaths even after larger doses of vitamin solution. The animals included in this study, however, had all shown residual symptoms for a considerably longer time than is possible without supplementation with vitamin B_1.

Symptomatology: Partial alleviation of the symptoms promptly occurred after administration of the thiamin chloride, except in a few animals which did not respond even after larger doses were given. The residual symptoms can be roughly grouped into two types. In the first type symptoms of impaired equilibration were manifested by difficulty in maintaining normal posture, by spasmodic and incoordinate movements of the head, deviation of the head to the right or left, or head retraction, and unilateral or bilateral deviation or protrusion of the eyeball. Loss of the vestibular righting reflex and convulsive attacks were also present to varying degrees and were usually continued throughout the experimental period. Such animals, if inadvertently cured by too large a dose of vitamin, showed a return of symptoms after a variable time had elapsed. Usually the attacks became progressively more severe and finally reached a stage where cure was impossible even when large (100 gamma) doses of thiamin chloride were given.

The second type of residual symptoms consisted, in its mildest form, of a peculiar ataxic hesitant type of walk which is not often seen in the acute condition. This condition was often present immediately after the vitamin was fed, but more frequently became apparent only some days after supplementation. More severe cases showed definite partial paralysis of one or both hind legs. Involvement of the forelegs occurred but this was infrequent. In most cases the condition became progressively but slowly worse; in a few animals the condition showed no increase in severity. Finally, a complete flaccid paralysis of one or more extremities

was often seen. Sensation in the affected extremity was impaired but not absent. Pricking with a needle or exerting pressure on the paw produced very little reaction. In some of the more severely affected animals subcutaneous edema of the affected extremity was noted. In fact, these animals showed considerable resemblance to those in which the sciatic nerve had been transected.

The equilibratory and the ataxic or paralytic type of symptoms were often present in the same animal, but they were also seen as separate manifestations in numerous instances.

It is of interest in connection with the supplementation with thiamin chloride to note that a prompt increase in food consumption and in weight occurred. Typical of this are the results obtained when an animal which had shown considerable residual symptomatology was given two 100 gamma doses of thiamin chloride in an attempt to cure the symptoms. This animal promptly increased its food consumption and gained 25 gm. during the succeeding week despite the persistence of the symptoms.

Gross Pathology: At autopsy the peripheral nerves of a few animals that had shown severe symptoms were observed to have localized enlargements along their course. The enlargements apparently had no particular site of distribution nor did they occur in the nerves of rats receiving any particular diet. In most of the animals no abnormalities were visible on gross examination.

Microscopic Pathology: Peripheral nerves examined by the polarized light method showed a variety of changes that were not present in the nerves of animals killed during the more acute phase of the deficiency. The changes were usually of a focal patchy type but occasionally were found to involve the whole nerve trunk.

In those nerves showing the mildest type of involvement there was a type of edematous change similar to but more severe than that seen in the nerves of animals showing the acute condition (Fig. 4). The myelin sheath, axis cylinder and interfibrillar connective tissue all shared in the edema. By uncrossing the Nicol prisms [10] periaxillar accumulations of isotropic material were observed. The myelin sheaths were frequently hazy but contained a few large droplets of isotropic material. However, in a few cases a definite granular appearance of the myelin was present which suggested the accumulation of minute droplets of isotropic material. The nodes of Ranvier were enlarged and dumb-bell shaped

and there appeared to be an excess of granular bubbly material which remained light at the points of extinction. There was an increase in the amount of isotropic material present in the incisures of Schmidt-Lantermann.

In the severely affected nerves the changes were apparently accentuations of the changes just described. The fibers became more enlarged, the axis cylinders became roughened, and more periaxillar isotropic material was present (Fig. 5). In still more severely affected cases some of the fibers became completely isotropic while others showed marked enlargement and very large bulbous areas along their course. In Figure 7 are seen the remnants of two fibers that are almost completely isotropic, while Figure 8 shows the results of uncrossing the Nicol prisms to determine whether these remnants are really isotropic fibers or merely small fragments resulting from uneven cutting of a fiber. Unless this procedure is resorted to one is unable to determine whether a blackened area seen with crossed Nicol prisms represents a space between fibers or a group of isotropic fibers. Figures 13 and 14 show the most severe type of degenerative change encountered in this study. These changes occurred in an animal that had shown a progressive degree of ataxia and paralysis for 57 days after the development of neuromuscular symptoms. The dumb-bell shaped enlargements seen in Figure 13 indicate that the effect is at the nodes of Ranvier. The marked enlargement will be better appreciated if Figure 13 is compared with Figures 1 and 2.

Nerves prepared by the sudan III method showed no evidence of the degenerative change so clearly and consistently depicted by the preparations examined with polarized light.

Discussion

It is apparent from the results of this study that the effect of an acute vitamin B_1 deficiency on the peripheral nerves is mild as compared with the effect of a more prolonged or chronic deficiency. In the acute deficiency even the sensitive polarized light method failed to reveal any changes other than a few fibers of Wallerian degeneration, such as were also seen in normal nerves, and a mild edema. The latter likewise occurred in the nerves of control rats receiving adequate vitamin B_1 but whose food intake was limited to that of the deficient rats. This limitation of food intake also

increased the incidence of Wallerian degeneration in the groups receiving vitamin B_1 as compared with similar groups not receiving the vitamin. It is believed that this was the result of a more severe inanition in the rats receiving the vitamin.

The chronic deficiency, however, produced marked changes of an edematous type in the peripheral nerves; the fibers were enlarged and in some cases showed large bulbous areas along their course, and there was a variable increase of isotropic material, some fibers becoming completely isotropic. It seems significant, in view of such marked changes in the nerves, that a stage is eventually reached where the symptoms cannot be cured even by the administration of relatively large doses of thiamin chloride. This, of course, might mean the lack of another essential factor of the vitamin B complex. The fact that the symptoms could be cured by thiamin chloride earlier in the history seems to indicate an eventual irreparable damage to the tissues rather than the lack of an additional factor. Moreover, rats receiving the same basal diets used in this study supplemented with thiamin chloride have shown no abnormal symptoms other than a slightly subnormal rate of growth in an experimental period of 18 weeks.[13] Gildea, Kattwinkel and Castle [14] have also reported that after repeated cures the process became irreversible and vitamin B deficient dogs were no longer cured by large doses of vitamin B concentrate.

Summary

1. The histopathology of the peripheral nerves of rats showing either acute or chronic vitamin B_1 deficiency has been studied by means of the polarized light and sudan III methods.

2. The nerves of rats that had developed symptoms of the acute deficiency showed only a few fibers of Wallerian degeneration, such as may be seen in normal nerves, and a mild edema.

3. The nerves of control rats receiving adequate vitamin B_1 but whose food intake was limited to that of the deficient rats likewise showed a mild edema and more Wallerian degeneration than was found in the nerves of the deficient rats.

4. Nerves of deficient rats that became moribund without developing typical neuromuscular symptoms showed more Wallerian degeneration than the nerves of those that developed neuro-

muscular symptoms, but less than the control rats receiving adequate vitamin B_1 with a limited food intake.

5. Residual symptoms consisting of disordered equilibration or paralysis occurred in rats in which a state of chronic deficiency had been produced by the administration of small amounts of thiamin chloride after the development of neuromuscular symptoms.

6. The chronic deficiency produced marked changes of an edematous type in the peripheral nerves. The fibers were enlarged, in some cases showing large bulbous areas along their course, and contained increased amounts of isotropic material; in the most severe cases some fibers were completely isotropic. These changes were demonstrable by the polarized light method but not by the sudan III method.

7. The rats eventually reached a stage where cures were no longer possible even by the administration of relatively large doses of thiamin chloride. The changes in the nerves indicated that this was the result of irreparable damage to the tissues.

Note: The authors wish to thank Miss Cornelia Stevens for aid in the preparation of the tissues and the microphotographs.

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DESCRIPTION OF PLATES

PLATE 45

All photographs except Figure 8 were taken between crossed Nicols at the point of greatest birefringence at a magnification of × 500. Wratten metallographic plates and a contrast developer (Eastman D–19) were used. All sections were cut at 10 microns.

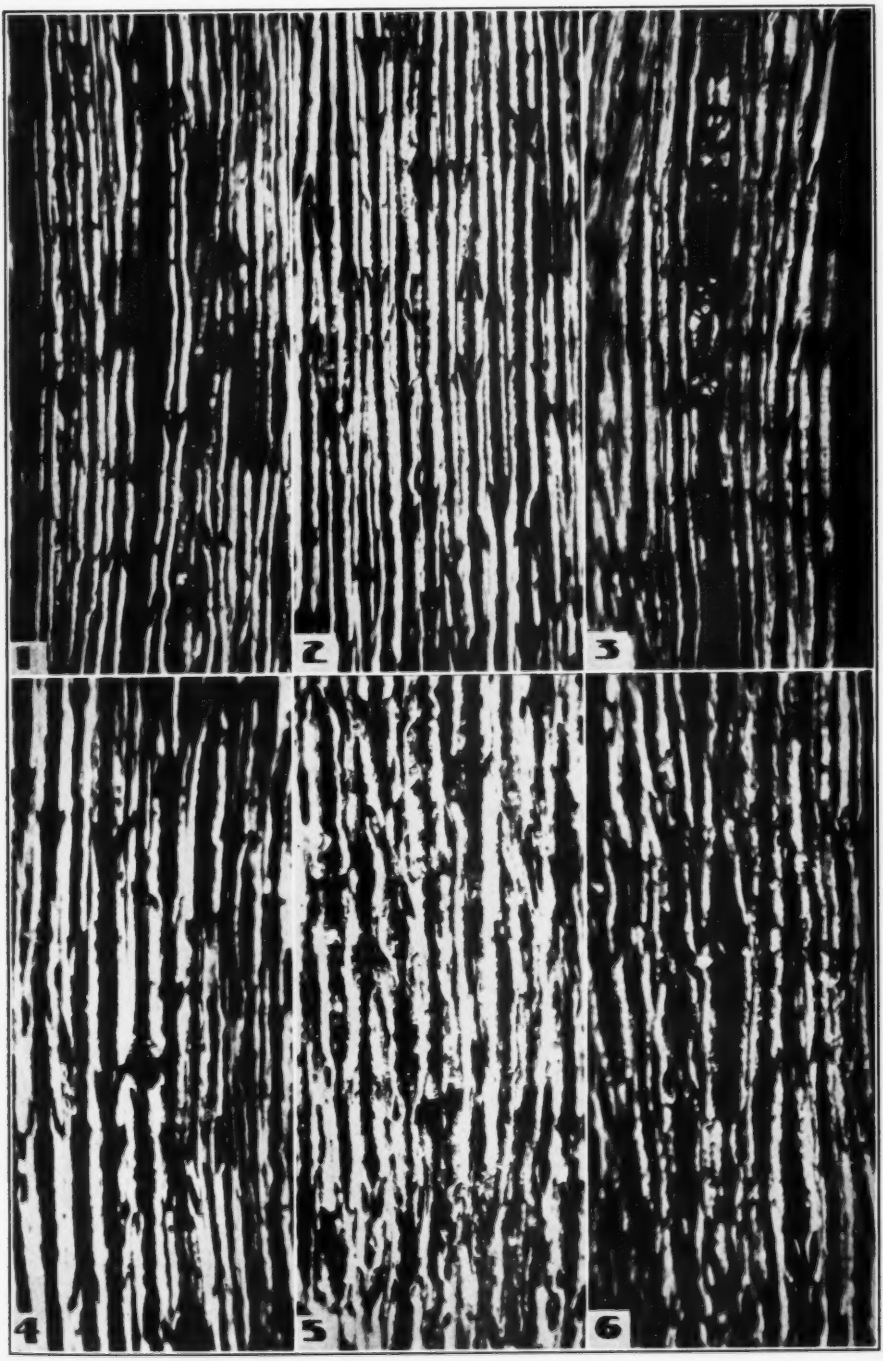
FIG. 1. Normal nerve. Showing the typical structure of normal fibers as shown by sections 10 microns in thickness. The darkened portions of the nerves have been shown by observation with uncrossed Nicols to be the results of tangential cutting and not to increased isotropic material.

FIG. 2. Acute deficiency. Showing the increased size of the fibers, the increase in isotropic material at the incisures of Schmidt-Lantermann, and occasional droplets of isotropic material in the myelin sheath. The borders of the axis cylinders are more ragged than normal and observation with Nicols uncrossed shows slight periaxillar accumulations of isotropic material at times.

FIG. 3. Control animal receiving limited (isocaloric) amounts of food plus ample vitamin B_1. Showing one of the numerous fibers in advanced stages of Wallerian degeneration. Note also the haziness and darkening of the myelin sheaths. In the upper left corner is a fiber showing an earlier type of change.

FIG. 4. Chronic deficiency. This type of change is common in the nerves of animals that have shown residual ataxia or symptoms of disordered equilibration. Note the increase in size of the fibers, the ragged axis cylinders, and enlargement of nodal structures.

FIGS. 5 and 6. Chronic deficiency. Showing a progressive increase in the severity of the myelin and axis cylinder changes in the nerves of animals showing definite paralysis.


1
2
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4
5
6

PLATE 46

FIG. 7. Chronic deficiency. Isolated fibers showing a moderately severe degree of change. The remnants of two fibers which are almost completely isotropic are seen. Compare with Fig. 8.

FIG. 8. Chronic deficiency. Photograph of the same fibers as shown in Fig. 7, taken with Nicols uncrossed. Note that the fibers seen as remnants in Fig. 7 are really still present within their sheaths. The wavy material represents interfibrillar connective tissue.

FIGS. 9–11. Chronic deficiency. Isolated fibers showing more severe changes in the size of fibers and presence of isotropic fibers. Note the nodal enlargements.

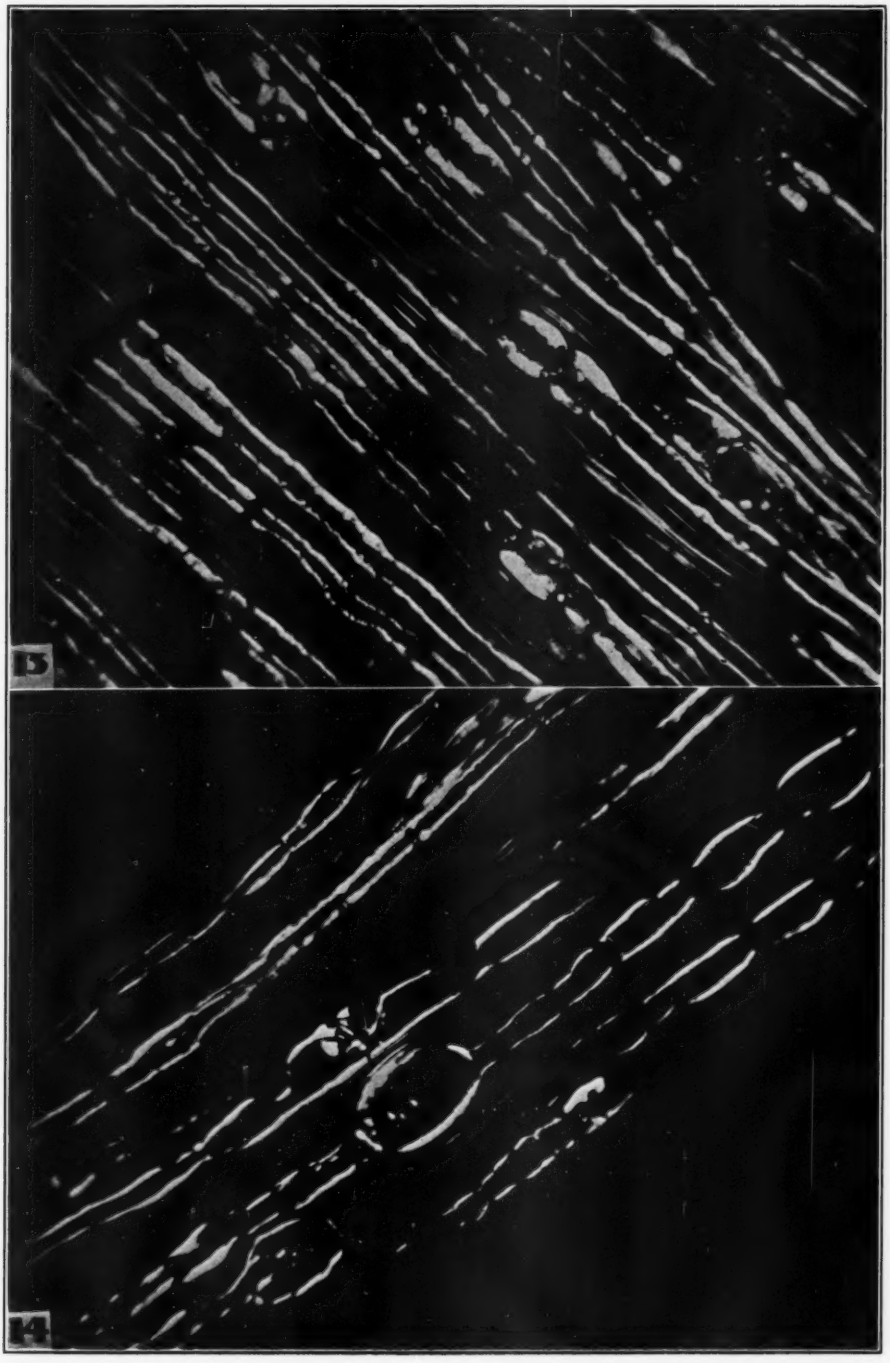
FIG. 12. Nerve from a chicken which had shown typical symptoms of neurolymphomatosis gallinarum, showing changes typical of advanced Wallerian degeneration.

Prickett, Salmon and Schrader Acute and Chronic Vitamin B_1 Deficiency

PLATE 47

FIG. 13. Chronic deficiency. Nerve showing a very severe type of edematous change. Note the marked enlargement of the fibers, particularly at the nodes, the presence of numerous fibers which are almost completely isotropic, and the focal nature of the changes.

FIG. 14. Chronic deficiency. Showing bulbous enlargement along the course of the fibers in nerves which are severely affected. Compare the size of the fibers with Figs. 1 and 2.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THE RESPONSE OF THE CENTRAL NERVOUS SYSTEM TO THE APPLICATION OF CARCINOGENIC HYDROCARBONS*

I. DIBENZANTHRACENE

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The possibility of producing tumors of the brain in experimental animals by the application of carcinogenic substances may be dated from the discovery in 1932 by Burrows, Hieger and Kennaway of the carcinogenic properties of 1:2:5:6 dibenzanthracene. Previous to this time considerable work on the carcinogenic properties of coal tar fractions had been accomplished. However, because of the high non-specific toxicity of such fractions, their application in almost all experiments was necessarily limited to the enduring skin. In this crude tar era there appears to have been only one attempt recorded [1] to apply such material to the brain of an experimental animal. The investigator injected an emulsion of tar into the subarachnoid space of rabbits by suboccipital puncture. It is alleged that the production of tar cancer on the ear and in the stomach was thereby accelerated. No mention is made of how the brain of the rabbit reacted to this treatment.

The introduction of dibenzanthracene, followed soon by a series of polycyclic hydrocarbons of high carcinogenic potency, immediately widened the scope of experimental cancer study. These chemicals, though exceedingly potent, are generally almost nontoxic, so that their application to internal organs and tissues becomes only a matter of interest and operative technique. Many experimenters were soon at work on such projects, and the diversity of tissues tested and results obtained may be found conveniently summarized in the comprehensive reviews by Cook and his colleagues.[2, 3]

The brain would seem to afford a particularly favorable organ in which to study chemical carcinogenesis. Here there are located

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in close juxtaposition a number of types of tissues of at least two embryological derivations. For their precise recognition there are available a number of excellent specific staining methods. This might be expected to make possible an accurate determination of the rôle of each of the various cell types entering into the formation of neoplasms. Observation of the common laboratory animals in large numbers for many years has shown that spontaneous brain tumors are practically non-existent, so that any positive results produced are immediately significant. Finally, brain tumors form a considerable and important division of human neoplasms; hence any anatomical or physiological information collected by experimental means might prove welcome and useful.

Judging from published reports, there has to date been little interest in attempting experimental carcinogenesis in the brain. In the course of a larger experiment, Ilfeld [4] in 1936 reported having inserted a pellet of cholesterol containing 5 per cent dibenzanthracene into the brain cortex of 2 stock mice. No results were obtained at the end of 8 months, but the number of animals used was manifestly too small for significance to be attached to negative findings.

Oberling, Guerin and Guerin [5] implanted crystals of benzpyrene beneath the pia and into the brains of 10 rats. No local reaction was seen, though only 3 animals survived the 10 months duration of the experiment. All these 3 exhibited pituitary adenomas — one each of chromophobe, spongiocyte, and castration cell type. One additional rat injected intracerebrally with a drop of 0.1 per cent benzpyrene in oil died in the 8th month with a tumor of epitheliomatous type invading the anterior lobe of the pituitary. No direct causal connection was clearly apparent in any case between the chemical applied and the tumor observed.

Athias [6] is apparently at present engaged in observing a series of 33 guinea pigs which he has injected intracerebrally with methylcholanthrene in arachis oil. He reported the occurrence in 1 animal of a "polymorphous cell sarcoma" in the auricle of the heart, but from his description the mass seems to be more likely only an organizing mural thrombus. He gave no results attributable to the action of the chemical in the brain.

Most recently Weil [7] has recorded an experiment on a small series of rats using dibenzanthracene, methylcholanthrene and

styryl 430. No tumors were obtained after 219 days in 3 rats injected with methylcholanthrene. Dibenzanthracene was implanted in 8 rats. Seven of these showed no neoplastic reaction after 229 days. In the 8th there was found a cystic mass lined by stratified squamous epithelium, located in the midbrain and apparently arising from the base of the skull. A direct causal relation between this mass and the implanted chemical was not demonstrated.

A compound, styryl 430, was injected into the brains of 5 rats. Two died soon afterward of injury or intercurrent disease. The remaining 3 survived up to 180 days. In all the latter there were found small cellular proliferations about particles of the chemical in the meninges and brain. While such aggregations undoubtedly contained individual cells resembling tumor cells, the appearance of the masses as a whole is more like an irritation hyperplasia than a true tumor. The masses were very small, there was no expansile or invasive growth, and no proliferation of blood vessels. This reaction falls far short of the aggressive malignant neoplasms which the carcinogenic chemicals are capable of producing in other tissues.

Technique

Following the earlier reported experiments, an attempt was first made to inject the dibenzanthracene in a 5 per cent suspension in lard. This material, however, proved too difficult to manage in the very restricted space available within the cranial cavity of the mouse. It was found on trial almost impossible to inject a small but accurately measured dose with a tuberculin syringe. The material welled out of the trephine wound into the subcutaneous tissues in a most undesirable fashion. Finally, in the few animals successfully injected, the lard vehicle produced a very confusing non-specific granulomatous lesion.

A solution to the problem of the vehicle of injection was furnished by Shear's studies.[8] He found that dibenzanthracene was readily soluble in molten cholesterol, and that pellets of 5 per cent concentration were fully as potent for carcinogenesis as the lard suspensions previously used. They possessed the further important advantage that they were not absorbed and dispersed, but remained recognizable in sections to mark accurately the

point of application of the hydrocarbon. Pellets were therefore prepared by drawing up a molten 5 per cent solution of dibenzanthracene in cholesterol into 1 mm. capillary glass tubes, oiled to facilitate removal of the solidified pellet. The finished pellet was a cylinder approximately 2 by 1 mm., of an average weight of 0.175 mg. and containing therefore about 0.072 mg. of dibenzanthracene.

It was at first hoped that for convenience anesthesia could be produced by an intraperitoneal injection of one of the drugs of the amytal group. This soon proved unsatisfactory because the depth of narcosis could not be controlled. The anesthesia was either so light that the animal struggled, or so deep that there was an unduly large operative mortality.

The technique of operation as finally developed was as follows: Anesthesia was induced by dropping the mouse into a jar containing a wad of cotton saturated with ether. As soon as the mouse lost consciousness, it was removed and placed on the operating table. This table consisted of an 8 inch square of heavy plank, to the center of which was attached a small L-shaped block of suitable size and height to support the animal's head. Anesthesia was maintained with ether on cotton in a small sputum bottle. The hair over the top of the head was roughly clipped, and the skin swabbed first with tincture of iodine and then with alcohol. The scalp was picked up with forceps, and with scissors an incision about 1 cm. long was made in the midline. The scalp was then pressed aside and the skull exposed by scratching away the galea aponeurotica with a dissecting needle. It is important to bare the skull thus, otherwise the soft tissues are caught by the burr with disastrous results. The skull was held firmly against the head block and a hole was cut in the parietal region by means of a 2 mm. spherical burr driven by an electric dental engine. There was usually little bleeding if the large vessel just behind the orbit was avoided.

Before commencing the operation the cholesterol pellets were placed in a small dish of 80 per cent alcohol. One was selected and placed in the tip of a small glass pipette 3 cm. long. The pellet was readily held in position by the capillary attraction of a small amount of the alcohol. The tip of the pipette was placed on the trephine hole, and the pellet pushed down into the brain by

means of a dissecting needle just long enough to expel the pellet from the pipette. The pipette was then laid aside, and the pellet adjusted with the needle so that its superficial end just appeared in the trephine opening. The scalp was drawn back into place and the incision tacked together with a single suture. The wound was finally dressed with a small amount of flexible collodion.

The above operative technique proved both rapid and efficient, and after a little practice the immediate mortality was less than 5 per cent. The animals recovered promptly from the operation and showed no apparent neurological defect. The dressings were usually clawed off in a week or 10 days, healing was sound and the hair was quickly restored. The animals were thereafter fed a good full diet and observed closely. Any animal appearing to be ill was isolated, because cannibalism was common and the eating usually began with the head.

Every precaution was taken to obtain ideal tissue for microscopic study. An animal appearing to be seriously ill was killed with illuminating gas, and the brain at once removed and placed in fixative. Autopsy technique was also the result of considerable experimentation during which some of the earlier brains were partially destroyed. The routine finally evolved was to pin the animal ventral side down and reflect the scalp by a midline incision. The operative field was inspected externally and the pellet identified. Usually the trephine hole was covered by a fibrous membrane through which the end of the pellet could be seen. The vertebral column was cut through with scissors as close as possible to the occiput. The occiput was then lifted up, and one blade of a small pointed scissors was thrust into the foramen magnum. Keeping close to the base of the skull the parietal bones were cut on both sides. A third cut across the frontal bones freed the calvarium. With a little care the whole brain and calvarium with the pellet undisturbed *in situ* could then be lifted away.

Without further manipulation the calvarium with brain attached was placed in Zenker's fluid containing 10 per cent of glacial acetic acid. Fixation for 24 hours in this solution not only afforded perfect tissue preservation but also completely decalcified the skull. After washing, the calvarium and brain were trimmed to a transverse slice 2 mm. thick containing the operative field and the pellet. This slice was embedded in paraffin in the

usual manner. Sections were routinely stained with hematoxylin-eosin, phosphotungstic acid hematoxylin, Masson's trichome stain, and Foot's modification of the impregnation method of Del Río-Hortega for reticulum. A few brains in which the pellet lay deeper and could be detached from the skull were fixed in formalin-ammonium bromide. It was found that frozen sections including the pellet could readily be cut, and specific impregnations such as the gold chloride-sublimate method applied to them. This technique will be employed more extensively in the cytological study to be published later.

Experimental Data

Using the operative technique described above, cholesterol pellets were successfully implanted in 81 stock albino mice, and 1 was injected with approximately 0.1 cc. of a 5 per cent lard suspension. The survival times and the number of living mice remaining at the end of each month are shown in Table I.

Table I

Time after operation	Number surviving	Time after operation	Number surviving
—	82	180 days	53
30 days	75	210 "	51
60 "	72	240 "	48
90 "	63	270 "	44
120 "	58	300 "	8
150 "	55	330 "	2

Forty-eight animals survived the period of 8 months found by Ilfeld [4] to be the average time required for the production of kidney tumors. Fifty-three lived for more than 6 months, the average interval calculated by Fieser [9] to be necessary for the formation of subcutaneous sarcomas.

In spite of the fairly severe trauma and the possible irritative action of the pellets, only 2 animals exhibited any recognizable neurological signs. These animals, dying 7 and 21 days after operation, showed contralateral extensor spasm and generalized convulsions of less than 24 hours duration. Most of the naturally occurring deaths seemed to be the result of pneumonia or gastroenteritis, and later of senility.

The implanted pellet was definitely identified at autopsy in all

but 8 mice. In the case of 5 of these the heads had been partly eaten and destroyed by cage mates. In the remaining 3 careful search failed to disclose any trace of the pellet. Probably it had been extruded during healing of the operative incision. Usually only the heads of dead animals were examined. A complete autopsy was done in only a few instances where the animal's condition suggested the presence of some interesting visceral disease.

It is not at present possible to describe in detail the sequence of tissue reactions to the implantation of the carcinogenic hydrocarbon. All effort in this first experiment was directed toward keeping as many animals as possible alive through the average incubation period of 8 months. Tissue examination was therefore limited to those animals dying naturally. Since such deaths occurred at irregular intervals, and since some of the earlier material was lost in the search for a suitable technique, the knowledge of tissue changes is very uneven. It is planned to fill in these gaps in subsequent experiments.

What was learned of the tissue reactions in this series was essentially as follows: Introduction of the pellet produced a laceration of the brain substance of considerable severity. More or less hemorrhage occurred about the pellet, and there was a prompt mobilization of microglia. These cells actively phagocytosed and removed the red blood cells and lipoid débris. This scavenger phase apparently lasted about 30 days unless prolonged by infection. Afterward there was left a residual small number of microglia distended with vacuoles of lipoid material. These remained up to the end of the experiment nearly a year later. A few microglia also assumed bizarre and elongated shapes and insinuated themselves into clefts between the crystals forming the pellet.

As early as 20 days after operation there could be demonstrated by silver impregnation a partial sheath of reticular mesodermal fibrils surrounding the pellet. The completeness and density of this sheath varied greatly in different specimens. A small portion of this mesodermal tissue proliferated from the vascular adventitia. The most of it was derived from the dura. The activity of the dura in this process was quite analogous to its well known activity in encapsulating hemorrhages. The pia and arachnoid seemed to react only feebly, if at all. In successful preparations

they could be seen to have been pushed down into the wound between the cortex and the encapsulated pellet with no apparent proliferation (Fig. 1). As expected, the densest encapsulation occurred in those specimens in which the pellet remained in contact with the dura. This was not invariably the case, though, and the degree of encapsulation was so capricious that it must be determined probably by the reactivity of the individual animal. The presence of such a capsule does not prevent the development of tumors. In fact, in the one tumor produced in this experiment, the capsule about the pellet was unusually dense and complete.

Reaction in the fixed nervous tissue was disappointingly mild during the interval of this experiment. Sometimes the ganglion cells receded slightly from the vicinity of the pellet. Gliosis was never marked, and less than one-half of the animals showed more than a minimum glial response. After about 4 months there were usually a few hypertrophied astrocytes with stout fibrils near the pellet. The glial reaction was always more pronounced in the white matter, and its degree was apparently not influenced by the extent of the mesodermal capsule about the pellet. In several specimens the pellet touched upon or penetrated through the ependyma of the lateral ventricle. In none of these was there any appearance of activity in the ependyma. It seemed merely mechanically stretched or displaced. When ruptured it occasionally everted and extended for a short distance outward along the surface of the pellet (Fig. 2). In a few animals portions of the choroid plexus lay in contact with the pellet but exhibited not the slightest morphological alteration. In these instances of contact or penetration of the pellet into the ventricle the cavity was often moderately dilated, but no explanation for this appeared in sections.

The reaction of the pia-arachnoid was correspondingly feeble. Usually it appeared merely stretched and ruptured by the pellet. In 8 specimens of 8 to 9 months duration there appeared small cellular thickenings of the arachnoid near the lips of the cortical wound, but not in direct contact with the pellet (Fig. 3). In minute structure such cell groups resembled the human meningioma, but they were exceedingly small and they showed no evidence of active proliferation or of pressure on adjacent tissues.

Accordingly for the present they do not seem to merit classification as true neoplasms.

Considering the crudity of sterile operative technique, there was relatively little evidence of serious meningeal infection observed in this series of animals. A chronic type of meningo-encephalitis, consisting of meningeal and perivascular round cell infiltrations with occasional focal parenchymatous lesions, was observed in 16 animals. In 6 of these such changes were slight, in 6 moderate, and in 4 marked. Since all degrees of this inflammatory lesion were observed to occur quite at random in mice dying at all stages of the experiment, it seems that the health of the animals was not seriously affected thereby. In only 1 animal a mixed fungus infection produced an abscess about the pellet and caused death on the 21st day.

The skull exhibited practically no reaction to the presence of the pellet. The trephine hole was closed by a dense acellular collagenous membrane derived indistinguishably from the dura and the periosteum. In the great majority of animals there seemed to have been little or no new bone formation and in no instance was bony healing complete. In some, the presence of one end of the pellet in contact with the trephine wound probably inhibited new bone formation. This mechanism, however, fails to explain the lack of bony healing in the fairly numerous animals in which the pellet was completely buried in the brain. In 1 animal killed 326 days after operation the entire calvarium was found to be increased to three times normal thickness. The bone showed a microscopic structure somewhat resembling that of Paget's osteitis in man. There was also partial ossification of the fascia covering the temporal muscles, and a lesser amount in the capsule surrounding the pellet. No evidence of inflammation appeared in adjacent tissues. As the condition was not recognized at the time of killing the animal, only the head was examined.

In this experiment only one tumor was directly caused by the carcinogenic chemical. This appeared in a male mouse found dead 282 days after operation. The tumor consisted of a moderately soft, hemispherical gray plaque of tissue 8 by 6 by 3 mm. firmly attached to the external surface of the skull over the point of inoculation. The scalp was intact and freely moveable over the tumor mass. Sections showed that the pellet lay obliquely

tangential, in contact with the brain, skull and periosteum. The pellet was apparently completely encapsulated, but tumor tissue had nearly destroyed the external half of the capsule. The tumor appeared to well up from the surface of the pellet through the trephine opening and spread over the skull beneath the scalp (Fig. 5). The outer table of the skull was partly eroded, and tumor tissue was beginning to invade the diploe. There was no definite intracranial extension, but for a short distance on either side of the trephine hole the dura was loose, cellular and infiltrated with a moderate number of round cells.

Histologically the tumor consisted of interlacing bundles of plump anaplastic spindle cells showing occasional mitoses (Fig. 4). It closely resembled the already familiar type of spindle cell sarcoma readily produced by the subcutaneous injection of dibenzanthracene. The presence in some fields of long, multinucleated, strap-like giant cells suggested a muscle cell origin. However, the definitely striated cells occurring in the tumor were clearly fragments of the partly destroyed occipitofrontalis muscle. By means of silver impregnation, reticulum could be seen to form a sheath about many tumor cells similar to that about muscle fibers. The tissue was unfortunately not sufficiently well preserved to permit the demonstration of myoglia fibrils. Blood vessels appeared as small thin-walled capillaries, or in the center of the mass more often in the form of irregular clefts between the bundles of cells. No hemorrhage or necrosis was seen. Small foci of lymphocytes were sparsely scattered in the tumor mass, and larger numbers were present along the advancing edge next the skull. The viscera showed no trace of metastases. Attached to the wall of the right auricle was an organized mural thrombus.

In this series of 82 animals 4 incidental neoplastic conditions were observed. None could be attributed to the experimental treatment. Two were the familiar type of spontaneous breast carcinoma, recognized 38 and 272 days after operation. A third tumor appeared as a large, rather soft and cellular mass beneath the angle of the jaw on the left in a male mouse 268 days after operation. Histologically this tumor was a rapidly growing small alveolar carcinoma, interpreted as arising probably from the thyroid. No regional or distant visceral metastases were found in any of these animals. The last neoplastic condition encountered

was an acute leukemia, which appeared in a male mouse 242 days after operation. Gradual enlargement of the abdomen had been noticed for 2 weeks before death, and the animal was carelessly assumed to be pregnant. It was found later moribund and killed, and the abdominal distention was discovered to be due to an enormously enlarged liver mottled with small yellowish patches of necrosis. Microscopically the liver, spleen, kidneys, adrenals and lymph nodes presented a massive leukemic infiltration. The heart and the vessels in the brain and other organs contained large numbers of immature leukocytes. These cells were large and non-granular, with round or indented vesicular nuclei, and closely resembled the blood monocyte. Unfortunately no blood or bone marrow studies were made because the nature of the condition was not recognized at the time of autopsy.

Summary and Conclusions

Cholesterol pellets containing 5 per cent of 1:2:5:6 dibenzanthracene were implanted into the brains of a series of 81 stock albino mice. The operative and histological technique employed has been described in detail.

Fifty-three of these 81 animals survived over a period of 6 months, the average interval shown by Fieser to be required for the production of subcutaneous sarcoma by means of dibenzanthracene. Histological observations indicated that the introduction of the pellets produced brain laceration followed by a marked phagocytic microglial response which disposed of the débris in about 30 days. A partial to complete collagenous capsule, chiefly from the dura, formed about the pellet. Thereafter a state of equilibrium between tissue and pellet seemed to be established.

On the 282nd day an extracranial, malignant spindle cell tumor was found, originating about the superficial surface of a pellet. In all, 40 animals survived longer than the period required to produce this tumor and were killed during the ensuing fortnight to end the experiment. In none of these did there appear any definitely neoplastic tissue reaction.

In the course of this experiment 4 spontaneous neoplastic conditions, 3 carcinomas and a leukemia, were encountered.

A dose of 1:2:5:6 dibenzanthracene known to be sufficient in

amount and duration of action regularly to produce subcutaneous sarcomas was applied to the brain and meninges. In this experiment one such sarcoma was produced about the exposed end of a pellet, but no neoplastic reaction was seen in the brains or meninges. It is therefore concluded that the brain and meninges of the mouse respond only very slowly or not at all to the carcinogenic stimulus of 1:2:5:6 dibenzanthracene.

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DESCRIPTION OF PLATE

PLATE 48

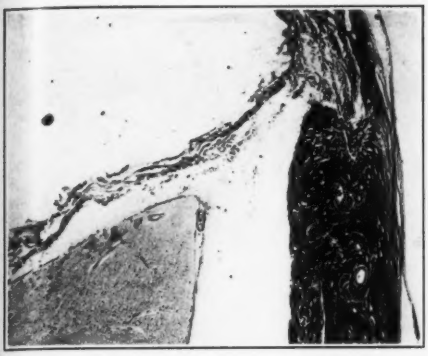
FIG. 1. Encapsulation of pellet by fibrous tissue proliferating from the dura and periosteum closing the trephine hole. Below this and quite separate, the delicate pia arachnoid covers the brain surface and passes down into the cortical wound. The pellet, dissolved during embedding, occupied the left upper third of the field. Foot-Hortega impregnation.

FIG. 2. Penetration of the pellet into the lateral ventricle with eversion of the ependyma. Phosphotungstic acid hematoxylin stain.

FIG. 3. Focal proliferation of cells on the external surface of the arachnoid in the vicinity of the pellet. Note the whorl formation on the right. Hematoxylin-eosin stain.

FIG. 4. Histological structure of the extracranial sarcoma shown in Figure 5. Two mitoses appear in the left center. Hematoxylin-eosin stain.

FIG. 5. Transverse section of brain and calvarium showing tumor mass arising from the external surface of the encapsulated pellet and spreading over the surface of the skull. To the right of the pellet the external table has been broken through and tumor is beginning to invade the diploe. In the right edge of the tumor are strands of the occipitofrontalis muscle. Masson's trichrome stain.



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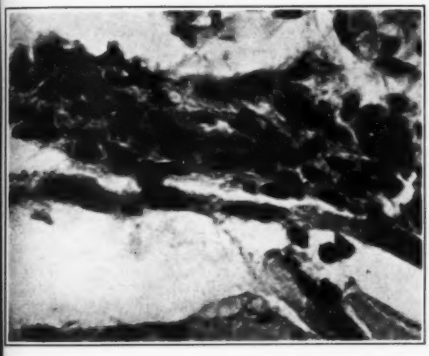

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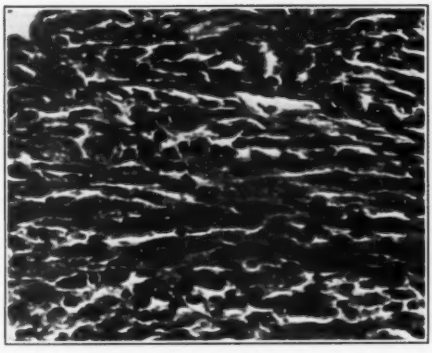

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A STUDY OF NORMAL AND RACHITIC BONE STRUCTURE BY MICROPHOTOGRAPHIC METHODS *

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INTRODUCTION

Since the discovery that vitamin D can prevent and cure rickets considerable work has been done on the mechanism involved and numerous studies of normal and rachitic bones by roentgenograms, silver impregnation methods, calcium estimations, and so on, have appeared in the literature. Microphotography has aided materially in these studies, not only by revealing structures visible through the microscope, but because of the sensitivity of plates to wave lengths beyond visible range, further structural and other details are also revealed. Recent improvements in photosensitive materials have furnished new and better instruments for the study of detailed structures. A series of microphotographs based on the radiation of different wave lengths (ultraviolet, normal (green), and infrared) on unstained bone sections are presented in this paper, illustrating how the penetrative powers of the wave bands employed affect the photographic plates differently.

EXPERIMENTAL METHODS

Normal, rachitic, and partially healed rachitic bones from rats were obtained by experiments carried out in the bioassay of the vitamin D content of foods. The method of assay was the U. S. P. XI, the Steenbock rickets-producing ration No. 2965 being used, and the extent of the rickets was determined by the line test on the corresponding bones of the same animals. The normal bones were from rats fed a normal diet and of the same age (55 to 60 days) as the rats with rickets. The femurs of normal, 2 plus healed (complete healing being represented by 4 plus), and rachitic rats were sectioned without preliminary decalcification. They were first embedded in celloidin and then cut in sections

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25 microns thick. The sections were mounted in glycerin under fused quartz coverslips on fused quartz microscope slides. The objective, eyepiece and substage prism of the microscope, as well as the slide and the coverslip of the preparation, were all of quartz, since ultraviolet light, to which ordinary glass is opaque, was one of the forms of radiation used. Even the walls of the cooling cell and the light filter were of quartz. An open automatic arc was used as the source of light in all the illustrations.

Since the object of this study was to compare the effect of ultraviolet, normal (green), and infrared wave bands on bone sections, the same optical system was used in all the microphotographs. All were taken at 18 diameters magnification.

To ensure negatives of comparable density, despite the different plates and filters necessary, the exposures required were worked out by the multiplicator method. This consists of taking a series of graduated exposures of strip photographs on the same plate. Leaving the light on continuously, the slide of the plateholder was only partially (½ inch) withdrawn and the shutter opened for an extremely short exposure. The slide was then opened to its next ½ inch strip and an additional short exposure made. This process was continued until a series of strip exposures varying from certain underexposure to certain overexposure was obtained. By changing the filter and plate, a strip exposure plate for the next series of photographs was prepared. In all, three such exposure plates were produced, one for each wave band employed. All were then tank developed simultaneously by the time and temperature method. Examination of the plates revealed sections correctly exposed and of comparable density. In all subsequent work these comparable exposures were used.

The microphotographs will be presented in groups of three for each bone section. Using the exposure data obtained in the strip multiplicator plates, three microphotographs in each group were made of the same section without removal from the microscope. The ultraviolet exposure was taken on a Hammer slow plate using a Schott and Gen. filter No. U. G. 2, which transmits light in the 3000 to 4000 Ångstroms wave band. The normal exposure was made on a Wratten "M" plate with a Wratten B2 filter, which passes light in the 4800 to 6000 Ångstroms wave band. The infrared was taken on an Eastman spectroscopic infrared plate 1P

with a Schott and Gen. filter No. R. G. 8, which transmits light in the 7300 to 9300 Ångstroms wave band.

In making the prints, all received the same exposure in the printing machine, and for each group the same time and temperature development was used. Thus, these microphotographs are as nearly comparable as it appears possible to make them under the given conditions.

Experimental Results

In Figures 1–9 are shown three groups of microphotographs of femurs of normal rats (Figs. 1–3), partially healed (2 plus) rats (Figs. 4–6), and rachitic rats (Figs. 7–9).

Examination of the three figures in each group reveals in each case a progressive clarity of detail and fineness of structure, especially in the epiphyseal layers, in progressing from the ultraviolet plate, with its excessive contrast, through the green to the infrared.

Dr. Clay R. Murray, associate professor of surgery at the College of Physicians and Surgeons, Columbia University, New York City, examined these microphotographs carefully. The following statement by him is presented with his permission:

"In examining the photographs so taken, both with and without the aid of a lens, it is readily evident that the definition of gross differences in the epiphyseal and para-epiphyseal regions, and in the epiphyseal plate in the three groups — normal, healing rachitic and frank rachitic — is much more marked in the infrared exposures than in either the normal or the ultraviolet exposures. The ultraviolet are less satisfactory than the normal (green band). In addition, there is discernible, it seems to me, in the infrared pictures a far greater number of structural and architectural details than in either of the other types of exposure, or in the bone viewed with normal light and a lens."

In order to determine whether the density of the ultraviolet prints obscured detail or not, a series of lighter prints was made from the plates of the bones of the normal and the rachitic rats. These are shown in Figures 10 and 11. It is evident that there is no improvement in structural detail in the lighter prints and that the relative superiority of the infrared plate as regards epiphyseal resolution remains.

Discussion

The most interesting result shown in the microphotographs is the greater detail evident in the infrared prints than in the ultraviolet and the normal light prints. There is not only a greater wealth of detail shown, but a better and sharper definition of all detail, as stated in the description of the experimental results.

In addition to the presentation of new material, a further discussion may perhaps be permitted. First, it was felt that the prints obtained based on the selective absorption of the bone elements would make it possible to study at leisure the finer structures of the various parts. Such a set of prints would not only differentiate morphological structures but they might also give further information in regard to the color and wave band absorption of the formed elements comprising the test material.

The present observations of the better resolution shown by the infrared light might perhaps be surprising if it were considered only that resolution increases with the numerical aperture of the lens, and also with the shortness of the light wave used.[1] The statement [2] (illustrated with microphotographs) is made in Photomicrography that "unstained whalebone sections appear as a black mass lacking in detail," when photographed with a blue filter, and further, that "the proper procedure is to photograph the object by the light which it transmits. The whalebone sections, for instance, photographed by red light give perfectly satisfactory results, showing ample detail in structure."

Bearing out these views and supplementing the evidence of the usefulness of the infrared studies, Clark [3] wrote: "Some rather opaque specimens show details of internal structure when photographed by the infrared which are not visible in ordinary photomicrographs." From a somewhat different point of view Rawling [4] stated: "Infrared photography has added considerably to the possibilities of microscopy. The advance is due almost entirely to the high transparency to the infrared radiation of many substances which are opaque to visible light. Thus, detail quite invisible to the eye may be disclosed by the infrared plate simply because infrared radiation penetrates the object so easily."

A statement of infrared radiation studies in the physiological field would not be complete without reference to the extensive

investigations of Massopust [5,6] in which various anatomical structures are presented in detail.

Further study of bone structure under varying conditions by the method of microphotography by infrared radiation are in progress.

Summary

A comparative microphotographic study of unstained sections of normal, rachitic, and partially healed (2 plus) rachitic bones of rats was made by the use of normal, ultraviolet and infrared radiation. Greater wealth of detail and better and sharper definition of all detail in the epiphyseal layers was obtained in the microphotographs made by infrared radiation. The technic used is described in detail.

Thanks are due Dr. Clay R. Murray for his advice and encouragement, especially in the interpretation of the data.

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DESCRIPTION OF PLATES

PLATE 49

FIG. 1. Section of femur of normal rat photographed by ultraviolet light (3000–4000 Ångstroms). × 18.

FIG. 2. Same section photographed by normal light (4800–6000 Ångstroms). × 18.

FIG. 3. Same section photographed by infrared light (7300–9300 Ångstroms). × 18.

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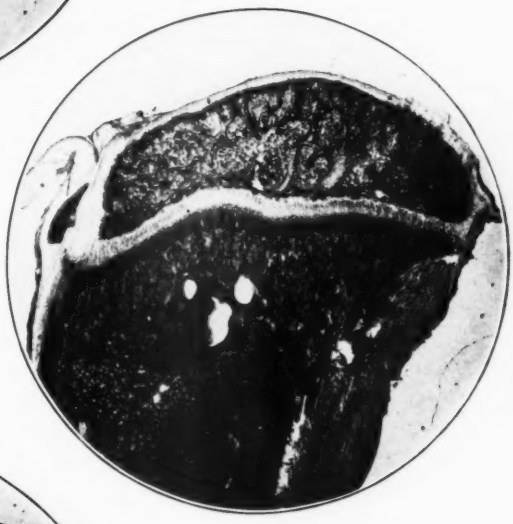

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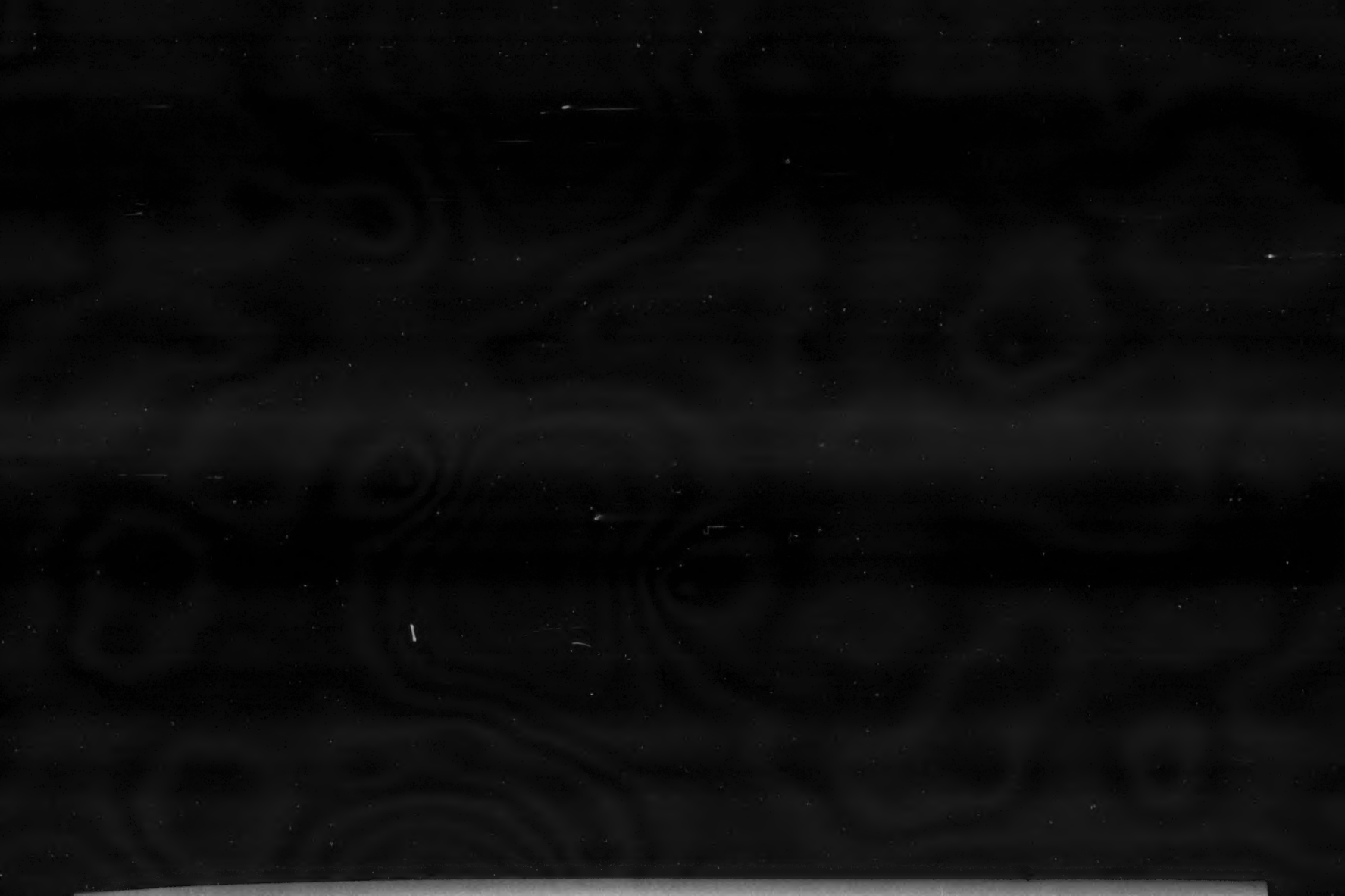
Siegel, Allen, McGuire and Falk Normal and Rachitic Bone Structure

PLATE 50

FIG. 4. Section of femur of partially healed rachitic rat. Ultraviolet light (3000–4000 Ångstroms). × 18.

FIG. 5. Same section photographed by normal light (4800–6000 Ångstroms). × 18.

FIG. 6. Same section photographed by infrared light (7300–9300 Ångstroms). × 18.



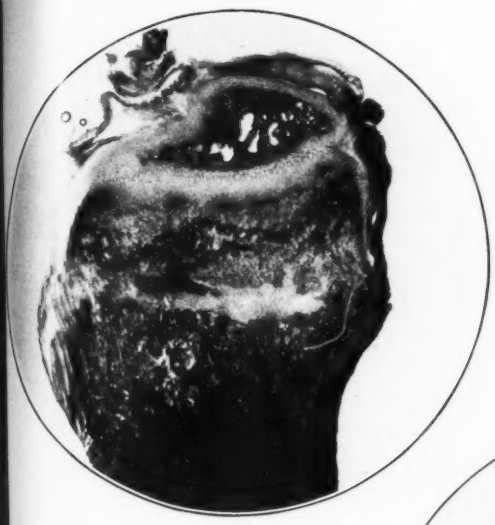


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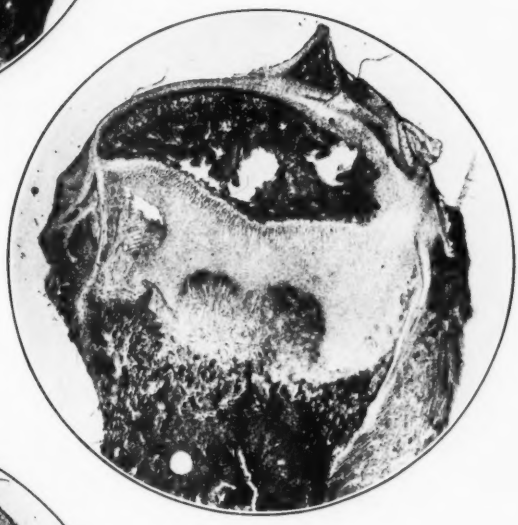
PLATE 51

FIG. 7. Section of femur from rachitic rat photographed by ultraviolet light (3000–4000 Ångstroms). × 18.

FIG. 8. Same section photographed by normal light (4800–6000 Ångstroms). × 18.

FIG. 9. Same section photographed by infrared light (7300–9300 Ångstroms). × 18.

7



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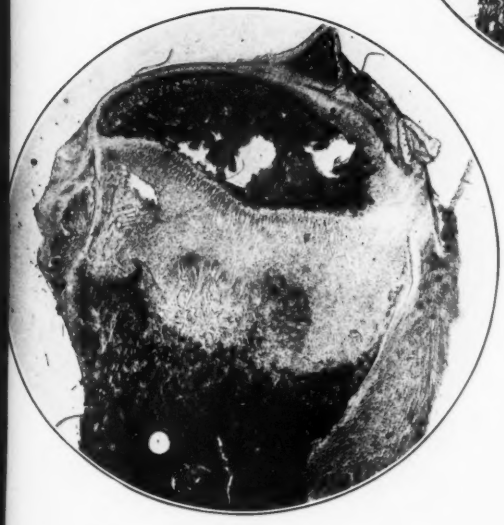

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PLATE 52

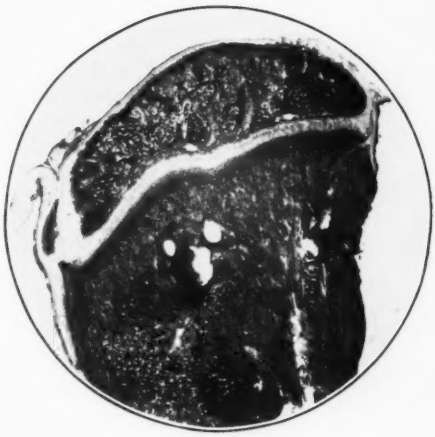
FIG. 10 a. Section of femur of normal rat photographed by ultraviolet light (3000–4000 Ångstroms). Light print.

FIG. 10 b. Same as Fig. 10 a. Dark print.

FIG. 11 a. Section of femur of rachitic rat photographed by ultraviolet light (3000–4000 Ångstroms). Light print.

FIG. 11 b. Same as Fig. 11 a. Dark print.

10 a



10 b

11 a

11 b

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Normal and Rachitic Bone Structure